STUDIES ON THE BIOCHEMISTRY OF TETRAHYMENA. X.
QUANTITATIVE RESPONSE TO ESSENTIAL AMINO ACIDS*

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Introduction.—Previous studies1–7 have shown that the ciliated protozoan, Tetrahymena, has nutritional requirements very similar to animals in general. This similarity is especially striking in regard to amino acid requirements where the ten essential amino acids of the dog, man, etc., have been shown to be essential also for Tetrahymena. Inasmuch as this protozoan possesses characteristics which make its culture in the absence of all other organisms relatively simple, it has been possible to investigate rather precisely the effects of various substances on its metabolism. Biochemical studies of this nature on other animals have been complicated and even vitiated by contaminating microorganisms of unknown synthetic abilities.

This report deals with the growth responses of Tetrahymena to varying concentrations of the essential amino acids and to serine, and includes the results of tests involving availability of natural and unnatural isomers.

Experimental.—The organism used in this investigation was Tetrahymena geleii W grown in pure (bacteria-free) culture. The general techniques have been previously reported.1, 4, 8 Dose-response curves were constructed for each of the essential amino acids, and for the stimulatory amino acid, serine, by adding graded amounts to a base medium containing the other ten in the proportions found in gelatin (table 1, medium II) together with growth factors, dextrose and salts. The minimum concentrations which permitted optimum growth under these conditions were combined, but the nitrogen level was then inadequate so these amounts were increased fivefold (medium III, table 1). Dose-responses were then studied using these new proportions. This medium was also used in studies on the availability of unnatural isomers.

The factor II preparations, which are necessary for the growth of this organism9, 10 were of two types. The one prepared from cerophyl, as
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AMINO ACID | MEDIUM II (MICROGRAMS PER ML) | MEDIUM III (BASED ON GELATIN) | MEDIUM III (BASED ON GROWTH OPTIMA)
---|---|---|---
L-arginine HCl | 820 | 125
L-histidine HCl | 100 | 125
DL-isoleucine | 350 | 125
L-leucine | 350 | 250
L-lysine | 600 | 250
DL-methionine | 340 | 500
DL-phenylalanine | 140 | 350
DL-threonine | 200 | 125
L-tryptophane | 100* | 50
DL-valine | 200* | 125
DL-serine | 40 | 250

* Amount arbitrarily added.

Both media contained the following substances (micrograms per ml.):

Dextrose | 1000
MgSO₄ . 7H₂O | 100
K₃HPO₄ | 100
CaCl₂ . 2H₂O | 50
FeCl₃ . 6H₂O | 1.25
MnCl₂ . 4H₂O | 0.05
ZnCl₂ | 0.05
Biotin (free acid) | 0.0005
Ca pantothenate | 0.10

Thiamine HCl | 1.00
Nicotinamide | 0.10
Pyridoxine HCl | 0.10
Riboflavin | 0.10
Pteroylglutamic acid | 0.01
Choline Cl | 1.00
Yeast nucleic acid (hydrolyzed) | 100.00

previously described,² was assayed quantitatively with Leuconostoc mesenterioides P-60, according to the method of Shankman, et al.,¹¹ and the following amounts of the ten essential amino acids were found (expressed in γ per ml. of final Tetrahymena medium): arginine, 0.9; histidine, 0.6; isoleucine, 0.0; leucine, 0.8; lysine, 0.2; methionine, 0.0; phenylalanine, 7.0; threonine, 0.0; tryptophane, 0.38; valine, 0.0. This preparation was satisfactory for all of the amino acids studied except phenylalanine. A factor II preparation which assayed 0.4 γ of phenylalanine per ml. of medium was used in the studies involving this amino acid. The starting material in this case was Liver Fraction L† (15 g.). This was dissolved in 750 ml. of water and extracted with butanol for 96 hours in a liquid-liquid extraction apparatus.⁸ After removal of the butanol by distillation in vacuo the volume was adjusted to 300 ml. and treated with Norit at pH 5.0. The final preparation was used in a concentration of 1:10.

All determinations were made on third serial transplants after 72 hours' incubation at 25°C. when serine was present, or 144 hours' incubation when serine was absent.

Results.—Serine.—As has been shown previously¹ serine is a general growth stimulator for Tetrahymena and this stimulation appears to be due to its ability to release inhibitions caused by the essential amino acids. It
will be shown later in this paper that serine plays a multiple rôle in growth, involving, together with the release of specific inhibitions, efficient use of certain of the amino acids. These relationships will be discussed with the specific amino acids concerned.

When serine is omitted from medium III no growth results but as little as 10 \( \gamma \) per ml. (table 2) of either natural or racemic serine provides maximum stimulation (range tested 0–250 \( \gamma \) per ml.). Both isomers are active. On the other hand, omission of serine together with a lowering of the concentration of any one of the essential amino acids (with the single exception of threonine) permits growth to occur, although at a greatly reduced rate and submaximal yield (table 3). This means that serine must be present to release the inhibition exhibited by each of the essential amino acids in the concentrations used. But the organism can partially replace the exogenous serine effect, provided that the concentration of at least one of the inhibitory amino acids is lowered.

**TABLE 2**

**Minimum Concentration Required for Maximum Response When Tested with All of the Other Amino Acids in the Concentrations of Medium III. Figures Represent Micrograms per Ml.**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>(Serine Present) L</th>
<th>(Serine Present) DL</th>
<th>(Serine Absent) L</th>
<th>(Serine Absent) DL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>20</td>
<td>...</td>
<td>0</td>
<td>...</td>
</tr>
<tr>
<td>Histidine</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>...</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>17.5</td>
<td>25</td>
<td>200</td>
<td>No growth</td>
</tr>
<tr>
<td>Leucine</td>
<td>25</td>
<td>140</td>
<td>25</td>
<td>140</td>
</tr>
<tr>
<td>Lysine</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Methionine</td>
<td>20</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>10</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Threonine</td>
<td>10</td>
<td>15</td>
<td>225</td>
<td>225</td>
</tr>
<tr>
<td>Tryptophane</td>
<td>10</td>
<td>12</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Valine</td>
<td>2.5</td>
<td>7.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serine</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Arginine and Valine.**—It was shown early\(^2\) that *Tetrahymena* could, under certain conditions, synthesize slowly both arginine and valine. Both of these amino acids are stimulatory but only in the presence of serine, which is to say that in the absence of serine growth is not improved when these amino acids are added to the medium at any level. The level giving maximum response for L-arginine is 20 \( \gamma \) per ml. (table 2) when serine is present, but no amount of added arginine stimulates growth in its absence (range tested 0–125 \( \gamma \)). Both natural and racemic valine were tested and, as in the case of arginine, no amount of either proved stimulatory in the absence of serine while maximum response was obtained, with serine present, with 2.5 \( \gamma \) per ml. of L-valine and 7.5 \( \gamma \) per ml. (table 2) of DL-valine (range tested L—0–35 \( \gamma \) per ml.; DL—0–250 \( \gamma \)). These quantita-
tive relationships indicate that only the natural form of valine is active for *Tetrahymena*, and that the d-isomer is somewhat inhibitory.

Tests were conducted to determine the cause of the failure of valine synthesis when tested with medium II. Additions of quantities of each amino acid to medium III so as to adjust its level to that of medium II showed that the high levels of arginine and lysine in the older mixture were responsible for blocking the synthesis of valine. Earlier observations\(^2\) have indicated that this block can be partially released by adding glycine, serine and cystine together.

### Table 3

**Summary of the Quantitative Relations Between Serine and the Essential Amino Acids**

Column I represents the concentrations in Medium III. Changes in concentrations are indicated in bold faced type.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
<th>X</th>
<th>XI</th>
<th>XII</th>
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<tbody>
<tr>
<td>DL-serine</td>
<td>250</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>L-arginine</td>
<td>125</td>
<td>125</td>
<td>0</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>L-histidine</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>7.5</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>DL-isoleucine</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>*</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>L-leucine</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>80</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>L-lysine</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>15</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>30</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>DL-threonine</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>225</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>L-tryptophane</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>10</td>
<td>50</td>
<td>10</td>
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<td>10</td>
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<tr>
<td>Growth†</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Neither lowering nor raising the concentration of DL-isoleucine permitted growth in the absence of serine, provided the concentrations of the other amino acids remained as in Column I. The substitution of 275 micrograms of L-isoleucine, however, permitted growth in the absence of serine.

† The plus sign (+) represents transplantable growth, the zero (0) represents no growth. The plus signs are not necessarily equal, as growth is slow and never maximum in the absence of serine, except where threonine levels are high (Column X).

**Histidine, Lysine, Methionine, Tryptophane.**—This group of essential amino acids will be considered together because they all behave similarly in relation to serine, although they differ in certain other details. In the absence of serine, growth occurred when the level of any one of these amino acids was lowered sufficiently (table 3) but the growth rate was low, as was the maximum yield. The maximum response levels were the same as those found in the presence of serine (table 2). Only natural histidine was available for testing and maximum response was obtained with the addition of 7.5 γ per ml. of medium (range tested 0–125 γ). Both isomers
of lysine are active. The maximum response was obtained at 15 γ per ml. of either L- or DL-lysine (range tested 0–300 γ). Both isomers of methionine appeared active, as the amounts required for maximum response were 30 γ and 20 γ per ml. of the DL- and L-form, respectively (range tested L—0–90 γ; DL—0–500 γ). This seems to indicate some inefficiency in the ability to metabolize the D-isomer. This condition is apparent also in the case of tryptophane where maximum response was obtained with 10 γ of L-tryptophane and with 12 γ of the racemic mixture (range tested L—0–20 γ; DL—0–50 γ).

**Phenylalanine, Leucine.**—When the concentration of phenylalanine is reduced in the absence of serine, low but transplantable growth results (table 3). The maximum response levels, either with or without serine, are identical but wide differences exist between the activities of the natural and racemic forms. Maximum response was obtained with 10 γ (table 2) of L-phenylalanine (range tested 0–140 γ) while 40 γ of the racemic mixture are required (range tested 0–350 γ). The activity of leucine is similar to phenylalanine. The maximum response levels either with or without serine were identical but, again, large amounts of the racemic mixture were required (25 γ of L-leucine; 140 γ of DL-leucine, range tested 0–700 γ). These large differences between the natural and racemic mixtures indicate competitive inhibition by the D-isomers and is found also in the case of valine, mentioned above. The natural form of leucine became inhibitory at levels above 125 γ per ml. when serine was absent but very high levels (700 γ per ml.) were tolerated when serine was present.

**Isoleucine.**—In the presence of serine both isomers of isoleucine appear to be metabolized, although the D-isomer somewhat inefficiently. Maximum response was obtained with 17.5 γ per ml. of the natural form and 25 γ per ml. of the racemic mixture (range tested 0–400 γ). In the absence of serine no growth was possible with any concentration of DL-isoleucine (range tested 0–400 γ). The D-isomer in this case appears to be completely inhibitory. Utilization of the L-isomer is faulty without serine as the growth was always slow, the yields low and the amounts required high (200 γ per ml.). These results indicate that serine, in addition to counteracting inhibition, makes isoleucine available for *Tetrahymena*. Unlike leucine, the natural form of isoleucine showed no inhibition without serine, in the range tested. Growth at levels of 400 γ per ml. was as good as at 200 γ per ml.

**Threonine.**—As with methionine, isoleucine and tryptophane, the unnatural isomer of threonine appears to be utilized (when serine is present) but not as efficiently as the natural form. Maximum response was obtained with 10 γ per ml. and 15 γ per ml. of L-threonine and DL-threonine (table 2), respectively (range tested 0–400 γ per ml.). In the absence of serine no growth occurred at these levels. Unlike the other amino acids,
high levels of threonine are not inhibitory but rather substitute for serine in the release of inhibition (table 3). The maximum yield with 225 γ per ml. of either natural or racemic threonine was as high as when serine was present, although, as in every case above noted, the growth rate was reduced. Release of inhibition apparently is not influenced by the stereoconfiguration of threonine any more than it is by serine.

Discussion.—The results of this study raise more questions than they answer. The complexity of the interactions between amino acids is perhaps not surprising when their general lability in metabolism is taken into consideration. Nevertheless, a number of the facts brought out should be considered if for no other reason than to attempt explanations which may be subject to experimental test.

Release of inhibition by serine requires some elaboration. It was stated that, under the conditions of these experiments, the reduction of any one of the amino acids except threonine reduced the total inhibition to a point where growth could occur without serine. An apparent exception to this statement was found with DL-isoleucine. No concentration of DL-isoleucine allowed growth to occur in the absence of serine, with low threonine, and the other amino acids high. It appears that non-inhibitory concentrations of isoleucine are insufficient for metabolic needs. Inhibitory concentrations of DL-isoleucine were always present (concentration of medium III, 125 γ per ml.) when the other amino acids were being tested, but this concentration was not enough to stop growth when their levels were reduced. It was obvious, therefore, that growth should occur at some level using DL-isoleucine if some other amino acid level were lowered. This was found to be the case. For example, if L-leucine was reduced from 250 γ to 80 γ per ml. and a dose response curve for DL-isoleucine constructed, it was found that high maximum yields were obtained with 25 γ per ml. of the latter. The growth rate was slow, as was expected in the absence of serine. High concentrations (350 γ per ml. and above) of the racemic isoleucine were completely inhibitory. The same general results were obtained when phenylalanine was reduced (from the 350 γ per ml. of medium III to 50 γ per ml.) or the threonine level raised (from 125 γ per ml. to 300 γ per ml.). Lowering of phenylalanine inhibition or raising the threonine concentration made even 400 γ per ml. of DL-isoleucine not completely inhibitory.

When one examines the summary given in table 3, one can see that growth resulted in the absence of serine, only when individual amino acid concentrations were lowered, with the single exception of threonine (Column X). That threonine can substitute for serine is perhaps understandable on the basis of their structural similarities.

In view of the fact that earlier work² had shown that glycine sometimes functions to antagonize inhibitions and that acetate in low concentrations
is stimulatory, and also that serine is readily converted to glycine, medium III minus serine was tested with graded amounts of glycine (range tested 0–200 γ per ml.). Again no growth occurred when only the ten essential amino acids were present, but growth did occur in the presence of glycine. Very low growth resulted with the addition of as little as 2.5 γ per ml. and steady increases occurred up to 50 γ per ml. Maximum yield at 50 γ per ml. was as high as when 10 γ per ml. of serine was added, but the growth rate was low, in contrast to serine. These quantitative relationships indicate that serine does not function by conversion to acetate via glycine.

In an attempt to improve growth in the absence of serine, and yet use the same isomeric forms of the amino acids as in medium III, a medium was constructed with the following concentrations (in γ per ml.): L-arginine, 40; L-histidine, 50; DL-isoleucine, 50; L-leucine, 100; L-lysine, 50; DL-methionine, 60; DL-phenylalanine, 50; DL-threonine, 300; L-tryptophane, 20; DL-valine, 7.5. The amino acids totaled 727.5 γ per ml. as compared to 2275 γ per ml. for medium III, and the amino nitrogen totaled 100.8 γ per ml. as compared to 308.6 γ per ml. for medium III. With this medium growth yields were nearly as great as when serine was added, but still the rate of growth was somewhat slow. Inhibition, as far as yield was concerned, was largely removed by the combined effect of high threonine and low concentrations of all of the other amino acids. The somewhat lower yield was possibly due to nitrogen limitations and the low growth rate due to faulty metabolism of isoleucine in the absence of serine.

Turning now to a consideration of the results on the utilization of the isomers, the most interesting and puzzling seem to be those with leucine, phenylalanine and valine. Even in the presence of serine more than double the amounts of the racemic mixtures than of the natural forms are required for maximum response. It appears that the D-isomers are inhibitory and unavailable but that this inhibition can be overcome, even though the 50–50 ratio of L to D is maintained, if larger amounts are added. One possible explanation is on the basis of competitive enzyme inhibition. At low levels of the racemic mixture 50% of the enzymes responsible for metabolism of the amino acid in question take up the available L-isomer and 50% D-isomer. As this binding of the enzyme by the D-isomer is reversible (according to the theory of enzyme competition) and the L-isomer is being used up, then at no time will the enzyme be able to mobilize enough L-isomer for adequate growth. As the concentration of both isomers is increased, however, there finally results a condition where 50% of the enzyme can at all times be saturated with the L-isomer, which, on the basis of margin of safety, is sufficient for optimum growth. If this is the true explanation for the results obtained with leucine, phenylalanine and valine, then we will have to assume enzyme action on some position of the molecule other than the asymmetric carbon, for these amino acids.
The apparent inability of this organism to metabolize efficiently the D-isomers of isoleucine, methionine, threonine and tryptophane cannot be explained at this time. In the case of methionine it may be that the less than double amounts of the racemic mixture as compared to the natural form for maximum response might point to the well-known double function of this amino acid: as an essential amino acid and as a methyl donor. As a methyl donor it may make no difference what the configuration is and therefore some of the D-isomer can be used to spare the L-isomer. Similar double functions, as yet unknown, may explain the results with the other three amino acids under discussion, i.e., niacin synthesis in relation to tryptophane.

**TABLE 4**

**Comparisons of the Rat, Mouse, Man and Tetrahymena Regarding their Ability to Utilize Optical Isomers of the Amino Acids. The Data for the Mammals Are Taken from a Similar Table in the Review by Albanece**

<table>
<thead>
<tr>
<th>AMINO ACID</th>
<th>Rat</th>
<th>Mouse</th>
<th>Man</th>
<th>Tetrahymena</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Histidine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Leucine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lysine</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Methionine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Threonine</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tryptophane</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Valine</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Serine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Inasmuch as *Tetrahymena*, unlike all other microorganisms so far studied under like controlled conditions, identifies itself with higher animals as far as amino acid requirements are concerned, it is interesting to compare the available data on other animals regarding isomer utilization. Table 4 was constructed from a similar one given by Albanece and details can be obtained from his review. In interpreting differences between the mouse, rat, man and *Tetrahymena*, it must not be overlooked that the intestinal flora of the vertebrates may play a decided rôle in the results obtained, while in this respect the data for *Tetrahymena* are more reliable. The results on the ciliate actually reflect its metabolism as all other organisms are excluded.

It has been found by Rose, *et al.*, using nitrogen balance studies, that histidine is apparently a dispensable amino acid for man. In contrast to this histidine is indispensable for *Tetrahymena*. No growth is possible when histidine is absent from the medium, but transplantable growth re-
results when as little as 2.5 γ per ml. are added, and 7.5 γ per ml. are sufficient
for optimum growth. With such an active substance, there is a strong
possibility that the bacterial flora of the human alimentary canal can con-
tribute enough to account for the results of Rose, et al. On the contrary,
under certain conditions Tetrahymena can synthesize valine. It is of
importance, in the light of this work, to test the ability of vertebrates to
dispense with valine under similar conditions of low arginine and lysine.

The results reported here, while incomplete in many respects, indicate
some important points, often overlooked in studies of this nature. Even
essential amino acids in moderate amounts can be inhibitory under certain
conditions. This may prove of importance in oral and intravenous
alimentation, especially with synthetic amino acids. The rôle of serine
as a growth stimulator, in its ability to release inhibitions and to enhance
the utilization of isoleucine, warrants further attention in metabolism
studies. That amino acid imbalance can influence growth and reproduc-
tion is again emphasized.

Summary.—1. Under certain defined conditions, the optimum concen-
trations of the essential amino acids were determined for the ciliated
protozoan Tetrahymena.
2. Availability of the optical isomers was investigated.
3. Quantitative results indicate that both the L- and D-isomers of lysine,
methionine, threonine and tryptophane are active. Both isomers of
isoleucine are active in the presence of serine.
4. The unnatural isomers of leucine, phenylalanine and valine are
inhibitory.
5. High levels of L-isoleucine are required in the absence of serine and
the racemic mixture is completely inhibitory.
6. Serine functions as an antagonist to the inhibitions exhibited by nine
of the essential amino acids.
7. In the absence of serine the growth rate was invariably low and the
maximum yield usually reduced.
8. High levels of threonine can substitute for serine for release of
inhibition but threonine is not a growth rate stimulator.
9. Comparisons are made between the data in the literature on verte-
brates and those reported here on Tetrahymena.

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Council acting for the American Cancer Society. A portion of this work was reported
at the Fourth International Congress for Microbiology in Copenhagen and an abstract
will appear in the Proceedings.
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‡ Unpublished results from this laboratory.
EFFECT OF INCREASING FOOD PROTEIN UPON THE CALCIUM CONTENT OF THE BODY*  

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In previous papers1, 2 we have noted briefly the fact that acceleration of growth by relatively high protein food may result in retardation of the body's normal developmental gain in calcium content. The purpose of the present paper is to record some further experiments bearing upon this relationship.

The experimental animals have been rats of like genetic background—an inbred laboratory stock of Wistar Strain albinos. The experiments are divided into two series according to the immediate nutritional background and the basal diet of the experimental animals.

First Series.—The experimental rats were separated at the age of 28 days from families fed Diet 16 (also called Diet A) consisting of five-sixths ground whole wheat and one-sixth dried whole milk with table salt in the proportion of 2% of the weight of the wheat. The air-dry food mixture contained practically 14% of protein and 0.2% of calcium. Food and water were constantly available to the animals. Rats of the same sex and essentially the same size were drawn from the same litters; in each case, one of these was continued on the basal Diet 16, while the other received Diet 16 plus casein. The casein was added in such proportion as to increase the protein content of the air-dry food mixture from approximately 14% to approximately 20%. At 60 or at 90 days of age the corresponding rats