TRANSAMINATION IN BACTERIA

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The enzyme-catalyzed transamination reaction involves the intermolecular transfer of an amino group from an α-amino acid to an α-keto acid. Since the discovery of this reaction by Braunstein and Kritzmann (1) in muscle tissue, the reaction has been studied in some detail, particularly in animal tissues (2, 3). More recently (4), a study of this reaction in germinating oat seedlings revealed the presence of a highly active transaminating system which was interpreted to play a direct rôle in protein synthesis. In contrast to the studies on plant and animal tissues, reports on the presence or absence of this reaction in bacteria have been equivocal. Thus, Adler et al. (5) stated that Bacillus coli catalyzed transamination but no experimental data were presented in support of this. Diczfalusy (6), working with B. coli and other organisms, reported negative results in a more elaborate study. On the other hand, Cohen (2) referred to unpublished data which showed a low but definite transaminase activity in B. coli. In view of the fact that bacteria are rapidly growing organisms, and thus must be capable of extremely rapid protein synthesis, it seemed important to study the transamination reaction in a variety of organisms. In this paper, experiments are reported which establish the presence in several species of bacteria of a potent transaminase system which catalyzes the following reaction.

(1) \( L(+)\)-Glutamic acid + oxalacetic acid \( \rightarrow \) α-ketoglutaric acid + \( L(-)\)-aspartic acid

Procedure

Preparation of Bacterial Suspensions—In general the desired organisms were cultivated on tryptose agar contained in Kolle flasks for 18 to 24 hours at 37°. For the streptococci and the pneumococcus, 0.1 per cent glucose tryptose agar was employed, while Bacillus welchii was cultivated anaerobically on the same medium in a Novy jar. Azotobacter vinelandii was grown in Burke’s synthetic medium at room temperature under aerobic conditions. After incubation the organisms were harvested, suspended in physiological salt solution, centrifuged, washed twice, and finally re-suspended in \( m/15 \) phosphate buffer solution of the desired pH. Total nitrogen content was determined on all suspensions by a micro-Kjeldahl method.
Preparation of Substrates and Incubation Mixtures—Glutamic and oxalacetic acids were added as neutral salts in concentrations of 0.12 M and 0.2 M respectively to give a final concentration of each of 0.021 M. The bacterial suspension and buffer solution were added to the main compartment of the Warburg cup, while the glutamic and oxalacetic acids were placed in the side arm in 0.5 ml. and 0.3 ml. amounts respectively. Inasmuch as it was quickly ascertained that transaminase activity could not be detected under aerobic conditions, all incubations were carried out anaerobically. Anaerobiosis was maintained by passing tank nitrogen through the cups. After 10 minutes shaking at 38° in a water bath, the cups were tipped, the reaction allowed to take place for the desired period of time, and then stopped by the addition of 1 ml. of 10 per cent sulfuric acid. The contents of the cups were washed into graduated centrifuge tubes, 1 ml. of 10 per cent sodium tungstate added, and the volumes read. The mixtures were then filtered, the volumes again recorded, and the solutions boiled for about 45 minutes to destroy any remaining oxalacetic acid. After adjusting to the original volumes with distilled water, an aliquot was taken for aspartic acid determination according to the method previously described (7). All determinations were carried out in at least triplicate. Rates of transamination are expressed as $Q_{TN}$ values.

$$Q_{TN} = \frac{\text{microliters aspartic acid formed}}{\text{mg. N} \times \text{hrs.}}$$

Results

Preliminary experiments were performed with suspensions of Bacillus coli with incubation periods of 1 and 2 hours, on the assumption that the previously reported negative results with bacteria may have been due to a very low transaminase activity. The results of these experiments indicated definite transaminase activity but of a low magnitude, i.e., 5 to 10 per cent transamination with $Q_{TN}$ values of 40 to 80. These values were in the same range as those previously found by Cohen (2). Further experiments, however, showed that Bacillus coli was able to deaminate and decarboxylate aspartic acid at such a rapid rate anaerobically that one could not expect any significant accumulation of this end-product. Thus it was found that of 1344 microliters of added aspartic acid, 45 per cent was destroyed in 5 minutes, and 75 per cent in 15 minutes under conditions simulating those employed in studying transamination. This effect is demonstrated in Fig. 1, in which it is seen that the apparent rate of transamination decreases with time. As a matter of fact, in the light of the rapid rate of aspartic acid disappearance, it can be seen from Fig. 1 that transamination is actually a very rapid reaction, and that the low rates observed after 30 or more minutes incubation are due to the rapid decarboxylation and deami-
nation of aspartic acid. That the $Q_{TN}$ values at even 5 minutes are not maximum is shown by experiments in which 1 minute incubation times were used. $Q_{TN}$ values as high as 3900 were obtained under these conditions. However, because of practical considerations, a 5 minute incubation time was employed in all the experiments reported.

Dilution experiments with increasing incubation times revealed that the same relative activity persisted in the system or systems responsible for deamination and decarboxylation, and consequently more dilute bacterial suspensions with longer incubation periods could not be employed to study the kinetics of this system.

Effect of Bacterial Concentration—The results shown in Fig. 2 indicate that transaminase activity in *Bacillus coli* is directly proportional to the concentration of bacteria within the limits of 1.95 to 7.8 mg. of bacterial nitrogen. In this range the $Q_{TN}$ values were quite constant with an average of 700. No appreciable transaminase activity was observed with a bacterial nitrogen content of 0.98 mg.

pH Optimum—It is seen from Fig. 3 that the pH optimum for the *Bacillus coli* system is about 8.5. This is essentially the same as that found for oat seedlings (4), but is higher than that reported for purified animal transaminase preparations (8). The latter had a pH optimum of 7.5. Previous to the determination of the pH optimum, all experiments were carried out at pH 7.6. Subsequent to this all experiments were run at pH 8. The optimal pH was not employed, since it seemed desirable to use a phosphate buffer solution.

Effect of Temperature—The optimal temperature for the *Bacillus coli*
transaminase system lies close to 32°C (Fig. 4). It is somewhat surprising to find that the rate of transamination decreases rapidly above this temperature. On the other hand, since intact cells were employed, it is quite consistent with the optimal growth temperature for *Bacillus coli*, which is in the neighborhood of 32°C. As can be seen from Fig. 4, between 10–32°C the rate of transamination is approximately doubled with each 10° rise in temperature.

![Figure 3](image1.png)  ![Figure 4](image2.png)

**Fig. 3**  **Fig. 4**

**Fig. 3.** Effect of pH on transamination with *Bacillus coli*; 2.63 mg. of bacterial nitrogen per flask; incubation time, 5 minutes.

**Fig. 4.** Effect of temperature on transamination with *Bacillus coli*; 2.76 mg. of bacterial nitrogen per flask; pH 8.0; incubation time, 5 minutes.

**Table I**

<table>
<thead>
<tr>
<th>Organism</th>
<th>$Q_{TN}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus coli</em></td>
<td>890</td>
</tr>
<tr>
<td>&quot; <em>dysenteriae</em> (Shiga)</td>
<td>685</td>
</tr>
<tr>
<td>&quot; <em>typhosus</em></td>
<td>1135</td>
</tr>
<tr>
<td>&quot; <em>proteus</em></td>
<td>1610</td>
</tr>
<tr>
<td>&quot; <em>pyocyaneus</em></td>
<td>800</td>
</tr>
<tr>
<td><em>Azotobacter vinelandii</em></td>
<td>1575</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>910</td>
</tr>
<tr>
<td>&quot; <em>albus</em></td>
<td>950</td>
</tr>
<tr>
<td><em>Bacillus welchii</em></td>
<td>1170</td>
</tr>
<tr>
<td><em>Streptococcus hemolyticus</em></td>
<td>865</td>
</tr>
<tr>
<td>&quot; <em>viridans</em></td>
<td>900</td>
</tr>
<tr>
<td><em>Pneumococcus</em> Type I</td>
<td>845</td>
</tr>
</tbody>
</table>

*18 to 24 hour cultures were employed; pH 8.0.*
QTN Values in Different Species of Bacteria—It appeared of interest to assay quantitatively different bacterial species for transaminase activity. All incubations were carried out for 5 minutes at pH 8 and 38°. While these conditions were as close to optimal as could be obtained practically for Bacillus coli, it is not at all certain that these conditions are optimal for the other bacteria investigated. Consequently, one must assume that the values reported in Table I are minimal. It is suggested from the data that different organisms within a species tend to have a transaminase activity of the same order of magnitude. This is particularly suggestive in the case of the staphyloccoci and the streptococci. Bacillus proteus and Azotobacter vinelandii show the greatest transaminase activity of the organisms studied. Since the latter is a nitrogen-fixing organism with high metabolic activity, the presence of a highly active transaminase system is of considerable interest.

DISCUSSION

The data presented in this paper provide unequivocal evidence for the occurrence of a highly active transaminase system in several species of bacteria. Diczfalusy's (6) failure to demonstrate transaminase activity in Bacillus coli with the following reactions

\[ (2) \ I(-)-Aspartic \ acid + \ \alpha\-ketoglutaric \ acid \rightarrow I(+)\-glutamic \ acid + \ \text{oxalacetic} \ acid \]
\[ (3) \ \alpha\-Ketoglutaric \ acid + I(+)\-alanine \rightarrow I(+)\-glutamic \ acid + \ \text{pyruvic} \ acid \]

is understandable, since both these reactions proceed very slowly as compared with Reaction 1 (9). As a matter of fact the rates of Reactions 2 and 3 would proceed so slowly that the end-products measured by Diczfalusy, viz., pyruvic and oxalacetic acids, would be destroyed by side reactions before they could accumulate in measurable quantities. Both these substances are rapidly utilized anaerobically by Bacillus coli and many other organisms. In the case of Reaction 2, which proceeds faster than Reaction 3 (9), the rapid deamination and decarboxylation of aspartic acid, demonstrated in the present paper, would preclude the measurement of any appreciable transamination under the conditions employed by Diczfalusy. On the other hand, glutamic acid is relatively inert, and had this end-product been measured, a small amount of transaminase activity might have been demonstrated at least for Reaction 2.

It is a matter of some interest to compare the relative rates of transamination in bacteria, higher plants, and animal tissues. In Table II some selected previously published values, converted to QTN values, for plant and animal tissues are presented. It is apparent that the highest QTN value obtained for Bacillus coli exceeds those found with animal tissues. Oat seedlings give the highest value of any unpurified preparation studied.
date, showing roughly one-half the activity of the purified heart muscle preparation. On the basis of the experiments with *Bacillus coli* it is more than likely that the values presented in Table I are 3 to 5 times lower than the optimal $Q_{TN}$ values. It can therefore be seen that most bacteria exceed animal tissues in their transaminase activity, and in some organisms such as *Azotobacter vinelandii* and *Bacillus proteus* the $Q_{TN}$ values obtained under optimal conditions would probably be several fold greater than those reported for animal tissues. Thus, in contrast to previous impressions, transamination is a more rapid reaction in bacteria and higher plants than in animal tissues. In view of the more rapid nitrogen metabolism and protein synthesis in these lower forms, such a relationship is quite understandable.

### Table II

$Q_{TN}$ Values of *Bacillus Coli*, Animal, and Plant Tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$Q_{TN}$</th>
<th>Bibliographic reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus coli</em></td>
<td>3,900</td>
<td>Present paper</td>
</tr>
<tr>
<td>Oat seedlings (96 hrs.)</td>
<td>5,650</td>
<td>4</td>
</tr>
<tr>
<td>Brain (rat)</td>
<td>2,800</td>
<td>9</td>
</tr>
<tr>
<td>Liver</td>
<td>2,200</td>
<td>9</td>
</tr>
<tr>
<td>Kidney</td>
<td>1,750</td>
<td>9</td>
</tr>
<tr>
<td>Heart muscle (rat)</td>
<td>3,330</td>
<td>8</td>
</tr>
<tr>
<td>Purified transaminase (beef heart muscle)</td>
<td>10,300</td>
<td>8</td>
</tr>
</tbody>
</table>

The data reported here are consistent with the view that transamination is in some manner directly concerned with the synthesis of protein (4). The $Q_{TN}$ values for the bacteria reported in this paper are considerably higher than any other metabolic quotients reported for these organisms. At least in the case of *Bacillus coli*, a search of the literature fails to reveal any metabolic $Q$ values on a nitrogen basis which approach the $Q_{TN}$ values given in this paper.

### SUMMARY

1. The transamination reaction

\[
\text{l(+)-Glutamic acid + oxalacetic acid} \rightarrow \text{\(\alpha\)-ketoglutaric acid + \text{l(-)-aspartic acid}}
\]

has been studied in several bacterial species. The optimal pH for this reaction in *Bacillus coli* lies at about 8.5, and the optimal temperature appears to be 32°.

2. $Q_{TN}$ values for twelve organisms are presented, all of which are of a high order of magnitude. Optimal $Q_{TN}$ values for *Bacillus coli* exceed those reported for animal tissues.
BIBLIOGRAPHY

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