Adenosine-5'-phosphate (AMP) and fumaric acid in the presence of an enzyme fraction purified from yeast autolysates condense reversibly to form adenylosuccinic acid (AMPS) (1).

\[
\text{HOOC-CH-OH_2-COOH} \\
\text{NH} \\
\text{N} \\
\text{N} \\
\text{ribose-5'-PO_4}
\]

Fumaric acid + AMP ⇌ ribose-5'-PO_4

This reaction is analogous to the aspartase reaction and to the enzymic condensation of arginine and fumaric acid (2). In this paper a more detailed characterization of the adenylosuccinase reaction and the product of the reaction, adenylosuccinic acid, is reported. A synthesis of the aglycone of adenylosuccinic acid, 6-succinoaminopurine, from aspartic acid and 6-chloropurine, which confirms the structure proposed for adenylosuccinic acid, is described in another paper.1

EXPERIMENTAL

Materials—The adenosine-5'-phosphate employed in these studies was a product of the Pabst Laboratories. Fleischmann's active dry yeast was the source of enzyme, and carboxyl-labeled fumarate was purchased from the Nuclear Instrument and Chemical Corporation.

Preparation of Adenylosuccinase—1 kilo of Fleischmann's active dry bakers' yeast was suspended in 3 liters of 0.1 N NaHCO_3 and vigorously stirred at 37°C for 5 to 7 hours. The mixture was frozen, thawed, and stirred at 37°C for 3 more hours. It was again frozen, thawed, and centrifuged at

* This work was supported by grants from the United States Public Health Service and the United States Atomic Energy Commission.
† Present address, Roswell Park Memorial Institute, Buffalo, New York.
3500 X g for 1 hour at 4°. The supernatant solution was collected and, for each 100 ml., 33 gm. of ammonium sulfate were added gradually, with stirring, at room temperature. The solution was stirred for an additional 30 minutes and then centrifuged at 3500 X g for 20 minutes at 25°. The supernatant solution was discarded and the precipitate dissolved in 400 ml. of distilled water. This solution was chilled to 4°, 80 gm. of ammonium sulfate were added slowly, and the solution kept at this temperature for an additional 30 minutes. After centrifugation at 3500 X g at 4° for 20 minutes, the precipitate was discarded, and 30 gm. of ammonium sulfate were added slowly to the supernatant solution, while the temperature of the solution was brought to 25°. After standing at this temperature for 20 minutes, the mixture was centrifuged (25°) for 20 minutes at 3500 X g, the supernatant solution discarded, and the precipitate dissolved in 100 ml. of distilled water.

At this stage of purification the preparation contained high fumarase activity which was removed by heat inactivation. Since there was some variability in the period of heating required to inactivate fumarase without inactivating adenylosuccinase, a preliminary small scale test was employed before heat treatment of the entire fraction. Samples of 0.1 ml. were kept at 60° in stoppered test-tubes for periods of 2 to 14 minutes, plunged into an ice bath, and diluted to 1 ml. with 0.1 m phosphate, pH 6.0. They were then clarified by centrifugation and assayed for fumarase activity (3) and adenylosuccinase activity (see below). The longest period of heating consistent with 90 per cent retention of adenylosuccinase activity invariably gave complete inactivation of fumarase. These conditions (usually 10 minutes at 60°) were then applied to the entire fraction and the denatured protein was removed by centrifugation. The supernatant solution at this stage (Table I) represents a 20-fold purification over the original yeast autolysate, with approximately 70 per cent recovery of activity in this fraction. The fraction was stable in the frozen state for several months. Further purification of adenylosuccinase activity may be achieved by application of the ammonium sulfate gradient elution technique (4) and by gradient elution from a column of calcium phosphate and cellulose, as described by Black and Wright (5).

Preparation of Adenylosuccinic Acid—By employing purified enzyme from yeast (Fraction IV), adenylosuccinic acid was prepared on a millimole scale by the following procedure. 1 mmole of adenosine-5'-phosphate and 9 mmoles of fumaric acid were dissolved in 50 ml. of distilled water by adjusting the pH of the solution to 6.0. 50 ml. of enzyme solution (Fraction IV dialyzed against 0.1 m phosphate, pH 6.5) were added, and the mixture was incubated at 37°. The progress of adenylosuccinic acid synthesis was followed at intervals by transferring 0.02 ml. of incubation
mixture to 5 ml. of 0.01 M NH₄OH to stop the reaction, and by determining the absorption at 280 mμ. During a 2 hour incubation period the absorption at 280 mμ increased approximately 3-fold as adenylosuccinate was formed. When equilibrium was reached, the solution was heat-treated (boiling water bath), and the denatured protein removed by centrifugation; the precipitate was washed with distilled water. To the combined supernatant solutions were added 1 ml. of concentrated NH₄OH and sufficient water to bring the final volume to 200 ml. This solution was then percolated through a column of Dowex 1-acetate, 2 per cent cross-linked, 200-400 mesh, which was 15 cm. long and had a diameter of 1.5 cm. The original effluent, containing no ultraviolet-absorbing material, was discarded, and the column was treated with a solution containing 2 M acetic acid and 0.25 M ammonium acetate until the absorption at 260 mμ of the effluent solution fell below 0.20. Usually 1000 to 1200 ml. of solution were required. This fraction contained residual adenylic and fumaric acids. A solution of 4.5 M ammonium acetate and 1.4 M acetic acid was then run through the column at the rate of 1 ml. per minute and collected in 10 ml. fractions. Absorption at 267 mμ was determined and fractions with optical densities lower than 10 were discarded. As determined by spectrophotometry, 0.725 mmole of adenylosuccinic acid was recovered in 50 to 60 ml. of effluent. The pooled fractions were concentrated to a syrup by vacuum distillation at 50° and 400 ml. of absolute ethanol were added with stirring. After standing 10 minutes at room temperature, the white precipitate was collected by centrifugation at 4° and the supernatant solution discarded. The precipitate was suspended with stirring in 400 ml. of absolute ethanol at 40° for 5 minutes and again collected by centrifugation. The precipitate in the centrifuge bottle was dried in a vacuum desic-
The yield of adenylosuccinate (ammonium salt) in several preparations averaged 300 to 350 mg., or approximately 0.6 mmole, and the purity varied between 88 and 95 per cent, as calculated from the phosphorus content. Analysis of the solid preparation of adenylosuccinic acid (by Huffman Microanalytical Laboratories) showed 4.12 per cent of ammonia nitrogen, which is equivalent to 1.43 moles of ammonium ion per mole of the nucleotide. According to the theoretical analysis for adenylosuccinate of this composition, N = 18.5, O = 36.2, C = 34.5, H = 4.45, and P = 6.35 per cent. The analytical results were as follows: N = 16.48 per cent; O = (method not applicable in presence of P); C = 34.07 per cent; H = 4.93 per cent; P = 6.35 per cent. The compound sintered at about 130° and melted with decomposition at about 155°; the melting point was not sharp. The analyses reported in Table II show that total phosphorus,

<table>
<thead>
<tr>
<th>Ribose, μmoles*</th>
<th>Total PO₄, μmoles†</th>
<th>5'-PO₄, μmoles‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.8</td>
<td>12.2</td>
<td>12.0</td>
</tr>
</tbody>
</table>

* Determined by the orcinol reaction.
† Total phosphate determined by the Fiske-Subbarow method.
‡ 5'-Nucleotide phosphate determined by enzymic hydrolysis with bull semen 5'-nucleotidase and estimation of inorganic P.

total ribose, and 5'-phosphate are present in equivalent proportions and indicate that the compound is a purine ribosyl-5'-phosphate. Attempts to crystallize adenylosuccinic acid or its salts were unsuccessful.

Chromatographic Behavior of Adenylosuccinic Acid—Although the ion exchange procedure described in the preceding section was found to be most suitable for obtaining solid preparations of adenylosuccinic acid, the nucleotide could also be separated from other nucleotides on Dowex 1-chloride. As shown in Fig. 1, AMPS is removed from the resin after adenosine diphosphate by a solution of 0.02 M HCl and 0.02 M NH₄Cl. If large amounts of inosinic acid are present, a preliminary treatment with 0.01 M HCl and 0.01 M NH₄Cl should be employed. The Rₚ values of adenylosuccinate on paper in isoamyl phosphate (7) and in butanol-acetic acid-water (2:1:1) are 0.98 and 0.28, respectively.

Titration Data for Adenylosuccinic Acid—A further purification of adenylosuccinic acid for electrometric titration was achieved by conversion of the compound to the ferric salt, decomposition of this salt through the cation exchange resin Dowex 50 in the hydrogen form, and neutralization
of the effluent with potassium hydroxide. All column operations were conducted rapidly to avoid the possibility of acid hydrolysis. The elec-

![Diagram](image)

**Fig. 1.** The separation of inosinic acid, adenosine diphosphate, and adenylosuccinic acid on a column 10 cm. long and 1.2 cm. in diameter of Dowex 1 resin, 200-400 mesh, 2 per cent cross-linkage, in the chloride form. The eluting agent was a solution containing 0.02 M HCl and 0.02 M NH₄Cl, and the flow rate was 0.5 ml. per minute. The tube volume was 8 ml.

![Diagram](image)

**Fig. 2.** Titration of 0.574 mmole of potassium adenylosuccinate with 0.1 N HCl. Water correction was made in accordance with the Debye-Hückel theory.

trometric titration of adenylosuccinate at the glass electrode is illustrated in Fig. 2. These data show that 4 equivalents of acid were consumed per mole in the titration between pH 9.0 and 1.8. Under these conditions,
the primary phosphoryl dissociation was not titrated and the four dissociations were assigned to the two carboxyl groups, the substituted amino group, and the secondary phosphoryl dissociation. The only deflection in the titration curve indicates a dissociation with pK 6.8, which is in the range expected for a secondary phosphoryl group.

Spectral Characteristics of Adenylosuccinic Acid—Depending upon the pH of the solution, adenylosuccinic acid had four distinct spectra (Fig. 3). The dissociations which influenced the absorption spectrum may be detected from a plot of absorbance at two wave-lengths against pH (Fig. 4).

![Fig. 3. Influence of pH on the ultraviolet absorption spectrum of 0.431 mM adenylosuccinic acid.](image)

One dissociation resulted in a change in absorbance at 267 but not at 280 μm, one resulted in a change at both wave-lengths, and one produced a change in absorbance at 280 but not at 267 μm. The dissociating groups had pK values of 2.3, 4.1, and 5.1, respectively. The last pK may be a high estimate, since there may be some overlapping of the last two dissociations.

By comparison with the pK values of aspartic acid and glutamic acid, the pK of 2.3 may be assigned to the α-carboxyl group. The pK values at 4.1 and 5.1 are believed to represent the dissociation of the secondary carboxyl group and the substituted amino group, though not necessarily respectively.

The molar absorbancy indices of adenylosuccinate at λmax at various pH values (Table III) were calculated from the phosphorus content by assuming that the compound contains 1 atom of phosphorus per molecule. It is
possible, however, to obtain the molar absorbancy index without reference to phosphorus analyses. During the degradation of adenylosuccinic acid

![Graph showing spectrophotometric titration of adenylosuccinic acid.](image)

**Fig. 4.** Spectrophotometric titration of adenylosuccinic acid. The arrows mark the approximate pK values of the dissociating groups.

### Table III

**Spectral Constants of Adenylosuccinic Acid**

<table>
<thead>
<tr>
<th>Solution</th>
<th>( \lambda_{\text{max}} ) (nm)</th>
<th>( \alpha_M \cdot \lambda_{\text{max}} \times 10^{-4} )*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 N HCl</td>
<td>266-267</td>
<td>16.9</td>
</tr>
<tr>
<td>0.05 M phosphate or acetate buffer, pH 3-3.5</td>
<td>266-267</td>
<td>18.5</td>
</tr>
<tr>
<td>&quot; 4.5-13</td>
<td>267-268</td>
<td>19.2</td>
</tr>
<tr>
<td>&quot; 7</td>
<td>267</td>
<td>19.9†</td>
</tr>
</tbody>
</table>

* \( \alpha_M \) = absorbance per gm. atom of P per ml. per cm.

† Calculated from the absorbance at the isoabsorptive point of the adenylosuccinase reaction (see the text).

by purified adenylosuccinase preparations, there was no change in the absorbancy of the solution at 259.5 m\( \mu \), indicating adenylosuccinate to be isoabsorptive with an equimolar mixture of adenylic and fumaric acids at pH 7. The molar absorbancy index of adenylosuccinic acid at 259.5 m\( \mu \) must therefore be \( 16.3 \times 10^3 \), the sum of the molar indices of adenylic acid \( (15.4 \times 10^3) \) (8) and fumaric acid \( (0.9 \times 10^3) \) (9) at this pH. Since
the ratio $\alpha_{267\text{ m}\mu}/\alpha_{239.5\text{ m}\mu}$ for adenylosuccinic acid at pH 7 was found to be 1.22, the molar absorbancy index at $\lambda_{\text{max}}$ under these conditions must be $1.22 \times 16.3 \times 10^3$, or $19.9 \times 10^3$. This value was in good agreement with that calculated from the phosphorus content.

Spectrophotometric Determination of Adenylosuccinase and Adenylosuccinic Acid—Adenylosuccinic acid was almost quantitatively converted to adenylic acid and fumaric acid by adenylosuccinase (Fig. 5, Curve A).

![Graph](http://www.jbc.org/)

**Fig. 5.** Synthesis and degradation of adenylosuccinate by yeast enzyme. Curve A, 3 ml. of $0.056 \times 10^{-3}$ M sodium adenylosuccinate in $0.05$ M phosphate buffer, pH 7, were incubated with $0.05$ ml. of purified yeast enzyme, and the absorbance at $280\text{ m}\mu$ was followed. Curve B, 3 ml. of a solution containing $0.050 \times 10^{-4}$ M sodium adenylylate and $4.5 \times 10^{-3}$ M sodium fumarate in $0.05$ M phosphate buffer, pH 7, were incubated with $0.05$ ml. of purified yeast enzyme, and the absorbance at $290\text{ m}\mu$ was followed. The concentrations of adenylosuccinate were calculated as described in the text.

This conversion resulted in a change in absorbance at $280\text{ m}\mu$ of 10.7 per micromole per ml., and a change in absorbance at $290\text{ m}\mu$ of 4.2 per micromole per ml. Adenylosuccinase activity was assayed from the rate of spectral change induced by the enzyme in a solution of $0.055 \times 10^{-3}$ M adenylosuccinate in $0.05$ M phosphate buffer. To 3 ml. of this solution was added sufficient enzyme solution to reduce the absorbance at $280\text{ m}\mu$ at the rate of about 0.1 per minute. At this level of activity the rate was constant for the first 2 or 3 minutes so that initial velocities were easily calculated. 1 unit of activity was defined as that amount of enzyme necessary to reduce the absorbance at $280\text{ m}\mu$ by 1.0 per minute.
Since the adenylosuccinase reaction was reversible (Fig. 5, Curve B), activity of the enzyme in catalyzing the synthesis of adenylosuccinic acid could be measured by employing a large excess of fumaric acid. The substrate solution for this assay contained 3 mM fumaric acid and 0.5 mM adenylic acid in 0.01 M phosphate, pH 5.9. A suitable quantity of enzyme was added to 3 ml. of this solution, and increments in the absorbance at 283 m\(\mu\) were measured against a blank consisting of substrate solution to which no enzyme had been added. If an amount of enzyme was used that increased the absorbance at the approximate rate of 0.08 per minute, a zero order reaction was obtained for the first 2 minutes.

The enzyme was used to identify adenylosuccinic acid in effluents from chromatography. For this purpose it was necessary to adjust the pH to 7.0 and the concentration to an absorbance at 280 m\(\mu\) of 0.2 to 1.35. A small quantity of enzyme was added to 3 ml. of this solution. If all the ultraviolet absorption was due to adenylosuccinic acid, the absorbance at 280 m\(\mu\) decreased to about 21 per cent of the initial value. It might be possible to employ adenylosuccinase for an assay of adenylosuccinic acid and adenylic acid in biological materials, but that was not attempted in this study.

**Table IV**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Micromoles</th>
<th>Specific activity, c.p.m. per (\mu)mole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Added fumarate</td>
<td>411</td>
<td>(1.17 \times 10^6)</td>
</tr>
<tr>
<td>&quot; AMP</td>
<td>52.5</td>
<td>0</td>
</tr>
<tr>
<td>AMPS formed</td>
<td>40.6</td>
<td>(1.11 \times 10^6)</td>
</tr>
</tbody>
</table>

The sodium fumarate and AMP were dissolved in 2.5 ml. of 0.1 M sodium phosphate, pH 5.75, and incubated with 20 ml. of enzyme (Fraction IV dialyzed against 0.1 M phosphate, pH 5.8) for 1 hour. The incubation mixture was heated for 2 minutes at 100° and the denatured protein was centrifuged and washed with 10 ml. of water. The combined supernatant solutions were chromatographed on Dowex 1-chloride as shown in Fig. 1.

**Enzymic Preparation of Carboxyl-Labeled Adenylosuccinic Acid**—Radioactive adenylosuccinic acid was prepared by the enzymic condensation of adenylic acid with \({}^{14}\)C-carboxyl-labeled fumarate, under the conditions given in Table IV. The conversion of adenylic acid to adenylosuccinic acid was 78 per cent and the specific radioactivity of the product was equivalent to that of the added fumarate. Since the fumarate was symmetrically labeled, both carboxyl groups must have been incorporated into adenylosuccinate.

The solution of radioactive adenylosuccinic acid from the Dowex 1-chlo-
ride column was rechromatographed on Dowex 1-acetate and precipitated as described above.

*Enzymic Degradation of C\(^{14}\)-Adenylosuccinate*—The enzymic degradation of C\(^{14}\)-labeled adenylosuccinic acid was carried out by incubating 0.8 \(\mu\)mole of the labeled compound with 0.1 ml. of adenylosuccinase (Fraction IV) in 0.4 ml. of 0.025 M phosphate buffer, pH 7. Immediately after the enzyme was added, 0.05 ml. of the mixture was transferred into 1 ml. of hot water and heated for 3 minutes in a steam bath. After 40 minutes at 30°, the remainder of the incubation mixture was heated; 0.1 ml. of each solution was run on a descending paper chromatogram in butanol-acetic acid-water (2:1:1), together with radioactive fumaric acid and malic acid for comparison. The chromatograms were scanned with a thin end window counter to localize and measure the radioactivity. All the radioactivity and ultraviolet absorption on the zero time sample migrated to the adenylosuccinate spot. After 40 minutes of incubation, chromatography showed 80 per cent of the radioactivity in the fumarate spot; adenylic acid was the only ultraviolet-absorbing component detected (Table V).

Acid Degradation of Adenylosuccinic Acid—Hydrolysis of adenylosuccinic acid in 1 n HCl at 100° for 15 minutes split the ribosyl linkage to yield an aglycone with an absorption maximum in acid at 275 to 276 m\(\mu\). The progress of this reaction, as followed by the spectral changes, is presented in Table VI. The compound has been isolated by paper and ion exchange chromatography and shown to be chromatographically identical with synthetic 6-succinoaminopurine.

Radioactive adenylosuccinic acid was hydrolyzed under these conditions and the hydrolysate chromatographed by descending chromatography in

### Table V

<table>
<thead>
<tr>
<th>Degradation procedure</th>
<th>Chromatographic spot</th>
<th>(R_F^*)</th>
<th>Net c.p.m.</th>
<th>Per cent total radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymic degradation</td>
<td>Initial AMPS</td>
<td>0.28</td>
<td>1296</td>
<td>80.4</td>
</tr>
<tr>
<td></td>
<td>Fumarate</td>
<td>0.84</td>
<td>1040</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>Adenylosuccinate</td>
<td>0.28</td>
<td>60</td>
<td>12.7</td>
</tr>
<tr>
<td></td>
<td>Unknown spot</td>
<td>0.45</td>
<td>164</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Adenylic acid</td>
<td>0.23</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>In HCl at 100° for 15 min.</td>
<td>Initial AMPS</td>
<td>0.28</td>
<td>199</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>Aglycone</td>
<td>0.52</td>
<td>156</td>
<td>0</td>
</tr>
</tbody>
</table>

* Descending paper chromatography in butanol-50 per cent acetic acid (1:1).
butanol-acetic acid-water (2:1:1). Only in the aglycone spot could radioactivity or ultraviolet absorption be detected. As shown in Table V, 78 per cent of the radioactivity was accounted for by the aglycone spot.

Properties of Adenylosuccinase—The purified enzyme solutions in 0.1 M phosphate at pH 6.5 were kept for several months in the frozen state with little loss of activity. Dialysis against distilled water decreased the stability of the enzyme. In solutions of high ammonium sulfate concentration (0.4 to 0.5 saturation) adenylosuccinase was more soluble at 0° than at room temperature.

Table VI

<table>
<thead>
<tr>
<th>Acid Hydrolysis of Adenylosuccinic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period of hydrolysis</td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td>min.</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>15</td>
</tr>
<tr>
<td>20</td>
</tr>
</tbody>
</table>

* This extinction coefficient is based upon the conversion of 1 mole of nucleotide to 1 mole of aglycone. Since a small amount of material may be destroyed during the period of acid hydrolysis, this value may be low. A solid preparation of pure aglycone derived by acid hydrolysis from AMPS has not been made, due to the high solubility of the free acid and its salts in aqueous and non-aqueous solvents.

Prolonged dialysis against 0.1 M phosphate buffer, pH 6.5, resulted in only a slight loss of activity. Ethylenediaminetetraacetate, in concentrations to 0.01 M, had no effect upon the activity of the enzyme.

The pH optimum for the degradation of adenylosuccinic acid by adenylosuccinase was found to be 7.0. The pH optimum for the reaction in the direction of synthesis was 5.9.

Equilibrium data were obtained from measurements of the spectral changes when adenylosuccinase was added to a solution of adenylic acid and fumaric acid (synthetic direction) or to a solution of adenylosuccinic acid and fumaric acid (degradative direction). When the change in absorbance ceased, more enzyme was added to insure that equilibrium had been reached. The concentration of adenylosuccinic acid formed or degraded was calculated from the equation

$$\Delta C = \frac{\Delta c_{280} \text{ m}\mu}{10.7}$$

where $\Delta C$ is the change in concentration of adenylosuccinate and $\Delta c_{280} \text{ m}\mu$.
is the change in absorbance at 280 m. In some experiments the substrate concentrations were too high for suitable measurements of an absorbance at 280 m, in which case the absorbance was measured at 290 m and the

**Table VII**

*Equilibrium Data for Adenylosuccinase Reactions*

<table>
<thead>
<tr>
<th>pH</th>
<th>Concentration, mm</th>
<th>Fumarate</th>
<th>AMP</th>
<th>AMPS</th>
<th>AMPS*</th>
<th>K_f × 10^3, moles per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0</td>
<td>2.23</td>
<td>0</td>
<td>0.0507</td>
<td>0.0133</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>6.75</td>
<td>2.23</td>
<td>0</td>
<td>0.0517</td>
<td>0.0121</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>2.23</td>
<td>0</td>
<td>0.0517</td>
<td>0.0125</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>4.50</td>
<td>0.100</td>
<td>0</td>
<td>0.0374</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>4.50</td>
<td>0.150</td>
<td>0</td>
<td>0.0588</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>4.50</td>
<td>0.375</td>
<td>0</td>
<td>0.152</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>4.50</td>
<td>0.750</td>
<td>0</td>
<td>0.288</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>0.300</td>
<td>0.75</td>
<td>0</td>
<td>0.0316</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>0.600</td>
<td>0.75</td>
<td>0</td>
<td>0.0581</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>1.20</td>
<td>0.75</td>
<td>0</td>
<td>0.106</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>2.25</td>
<td>0.75</td>
<td>0</td>
<td>0.176</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>4.50</td>
<td>0.75</td>
<td>0</td>
<td>0.283</td>
<td>7.0</td>
<td></td>
</tr>
</tbody>
</table>

Mean .......................................................... 6.8

* The quantity of AMPS formed or degraded was determined as described in the text. The equilibrial concentrations of the other reactants were obtained by subtraction from the initial concentrations.

\[ K_f = \frac{\text{fumarate/AMP}}{\text{AMPS}} \] at equilibrium; 35°.

Since the publication of the preliminary report of adenylosuccinase (1), the equilibrium constant has been determined again with more purified enzyme preparations, under a variety of concentrations of the reactants, in 0.05 M sodium phosphate, pH 6 to pH 7, at 35° (Table VII). The average value was found to be 6.8 × 10^{-3}. The method of Lineweaver and Burk (10) was used to determine the Michaelis constants \((K_m)\) for each reactant in 0.05 M sodium phosphate, pH 7.0, at 35°. The \(K_m\) for adenylic acid was determined in the presence of sufficient fumarate to saturate the enzyme (4.5 mM), and the \(K_m\) for fumaric acid was determined in the presence of sufficient adenylic acid to saturate the enzyme (0.75 mM). The \(K_m\) values
were found to be adenylosuccinate $1.2 \times 10^{-6}$ M; adenylate $4.8 \times 10^{-6}$ M; fumarate $5.2 \times 10^{-4}$ M.

Adenine, adenosine, adenosine-2'-phosphate, and adenosine-3'-phosphate would not serve as substrate for adenylosuccinase. Deoxyadenosine-5'-phosphate, however, was a substrate for the enzyme, as measured by the spectrophotometric assay of activity. No spectrophotometric or chromatographic evidence for a reaction of adenylic acid with maleic, oxalacetic, $\alpha$-ketoglutaric, malic, or malonic acid was obtained, nor did these compounds show inhibition of splitting or synthesis of adenylosuccinic acid.

DISCUSSION

The structure proposed for adenylosuccinic acid (1), 6-succinoaminopurine ribosyl-5'-phosphate, is supported by enzymic evidence for a 5'-nucleotide, and by the degradation of the compound synthesized from radioactive fumarate to an aglycone containing all the radioactivity of the parent compound. More definitive evidence for this structure has been obtained by synthesis, from 6-chloropurine and aspartic acid, of 6-succinoaminopurine, chromatographically identical with the aglycone derived from adenylosuccinic acid.

The rôle proposed for adenylosuccinic acid in purine biosynthesis as the immediate precursor of adenylic acid (1, 11) has not yet been established. However, Abrams and Bentley (12) found the transformation of inosinic acid to adenylic acid to be specifically dependent upon aspartic acid in a soluble enzyme system extracted from bone marrow. The functional analogy of adenylosuccinic acid with argininosuccinic acid is suggested in the following representation of the terminal steps in adenylic acid biosynthesis:

(1) IMP (or precursor) + aspartic acid $\rightarrow$ adenylosuccinic acid
(2) Adenylosuccinic acid $\Leftrightarrow$ AMP + fumaric acid

SUMMARY

Adenylic acid and fumaric acid were enzymically condensed to form adenylosuccinic acid by an addition reaction of the aspartase type. The rates of enzymic synthesis and degradation of the compound were determined by differential spectrophotometry. Adenylosuccinic acid has been prepared on a millimolar scale by this enzymic reaction and ion exchange chromatography. The titrimetric and ultraviolet absorption properties of the compound have been determined. These studies, and chemical and enzymic degradation, support the proposed structure of adenylosuccinic acid as 6-succinoaminopurine ribosyl-5'-phosphate. It is suggested that adenylosuccinic acid functions in purine biosynthesis as the immediate precursor of adenylic acid, a rôle analogous to that of argininosuccinic acid in arginine biosynthesis.
Addendum—Lieberman (13) has reported the synthesis of adenylosuccinic acid from inosinic acid and aspartic acid by a purified *Escherichia coli* enzyme which catalyzes the following reaction:

$$\text{IMP} + \text{aspartic acid} + \text{guanosine triphosphate} \rightarrow$$

$$\text{adenylosuccinic acid} + \text{guanosine diphosphate} + P$$

**BIBLIOGRAPHY**

THE PREPARATION AND PROPERTIES
OF ADENYLOSUCCINASE AND
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