Microcalorimetric Study of *Escherichia coli* Aerobic Growth: Theoretical Aspects of Growth on Succinic Acid

Z. DERMOУN†* AND J. P. BELAICH

Laboratoire de Chimie Bactérienne, Centre National de la Recherche Scientifique, 13274 Marseille Cedex 2, France

Two methods of investigation were used to evaluate the heat quantity associated with anabolic processes \( q_{an} \) during the aerobic growth of *Escherichia coli* in a minimal medium containing succinic acid as the sole energy and carbon source. The study of the contribution of biosynthetic reactions from succinic acid and ammonia were investigated by both methods. The two \( q_{an} \) values obtained were in excellent agreement and were found to be significant. Thus it was demonstrated that the contribution of anabolism strongly influenced the quantity of heat associated with microbial aerobic growth. The \( q_{an} \) calculated as above explained the experimental enthalpy change which was recently reported.

The enthalpy change associated with the aerobic growth of *Escherichia coli* in a minimal medium containing a limiting concentration of succinic acid as energy and carbon source was measured recently (3), and the value obtained was found to be significantly lower than that of the heat corresponding to the catabolic reactions. The difference between these values was attributed to the enthalpy change associated with reactions of biosynthesis.

Despite a great deal of work, no clear data are available concerning the contribution of anabolism to the experimental enthalpy change accompanying the microbial growth.

Recently, Belaich (in A. E. Beezer [ed.], *Biological Calorimetry*, in press) proposed two methods for evaluating enthalpy changes associated with anabolic processes \( (\Delta h_{an}) \) based on the heat of combustion of whole cells and on the scrutinization of the anabolic reactions step by step.

It was of interest to us to investigate the influence of the biosynthetic reactions on the enthalpy changes associated with the growth of *E. coli* in a minimal medium as measured by microcalorimetry.

**MATERIALS AND METHODS**

**Bacterial strains and media.** Bacterial strains and media were as described previously (3).

**Microcalorimetric technique.** The heat from combustion of the *E. coli*-grown cells was determined by Dr. Ducros (ENSTA). For this purpose, the culture was maintained at 30°C on a minimal medium containing a limiting concentration of succinic acid. The cells were harvested at the beginning of the stationary phase and washed twice with cold distilled water; then lyophilized samples of 1 g each were analyzed by combustion in an oxygen bomb calorimeter. The heat from the combustion of each sample was calculated, taking into account the water content of the lyophilized cells, and expressed in kilojoules per gram of cells (dry weight).

The bacterial formulation used in the text has been published previously (3). This formulation corresponding to 22.54 g of cells (dry weight), was \( \text{CH}_{1.56}\text{O}_{0.30}\text{N}_{0.23} \).

**RESULTS**

The heat from the combustion of succinic acid-grown bacteria as analyzed by the oxygen bomb calorimeter was found to be \(-22.9\) kJ/g. With these data and the cell formulation in hand an evaluation of the enthalpy of bacteria formation \( (\Delta h_f) \) could be done. This value was calculated by using a classical relationship related to cellular combustion and corresponds to the following: \( \text{CH}_{1.56}\text{O}_{0.30}\text{N}_{0.23} + 1.21\text{ O}_2 \rightarrow \text{CO}_2 + 0.78\text{ H}_2\text{O} + 0.115\text{ N}_2 \).

The enthalpy of formation of bacterial cells was found to be \(-4.42\) kJ/g or \(-4.42 \times 22.54 = -99.68\) kJ for the entity described as bacterial formulation.

The relationship corresponding to the bacterial synthesis of succinic acid and its nitrogen source may be written as follows:

\[
\text{CH}_{1.56}\text{O} + 0.23\text{ NH}_2\text{OH} \rightarrow \text{CH}_{1.56}\text{O}_{0.30}\text{N}_{0.23} + 0.54\text{ H}_2\text{O} + 0.16\text{ O}_2 \quad (1)
\]

The enthalpy change \( (\Delta h) \) corresponding to equation (1) was calculated based on the enthalpy of formation of all the compounds (and on the \( \Delta h \) associated with the dissolution of oxygen in water). This heat quantity, found to be \(+60.31\) kJ, was associated with the synthesis...
of 22.54 g of cells (dry weight) from 0.25 mol of succinic acid and 0.29 mol of ammonia. However, it was more useful to determine the enthalpy as \( \Delta H_{an} \), which was previously described and expressed as kilojoules per gram of cells (dry weight) (Belaich, in Biological Calorimetry, in press). For the anabolic reaction described by this relationship (1), the \( \Delta H_{an} \) was found to be +2.67 kJ/g. Therefore, it became easy to calculate the heat quantity associated with the anabolic process \( (q_{an}) \) during the metabolism of 1 mol of succinic acid. If \( Y \) is the molecular growth yield of \( E. coli \), with succinic acid as energy and carbon source, and if \( \alpha \) is the molar fraction of the succinic acid incorporated into the cellular material, this value could be written as follows:

\[
q_{an} = Y \cdot \Delta H_{an} = \alpha \Delta H_{an}
\] (2)

According to this equation, \( \Delta H_{an} \) was the enthalpy change corresponding to equation (1) when 1 mol of succinic acid was fully transformed into cellular material. For aerobically grown succinic acid cells, this last value was 60.31 \times 4 = 241.24 kJ/mol. Consequently, the heat quantity \( (q_{cat}) \) associated with the fraction \( (1 - \alpha) \) of the succinic acid oxidized during the metabolism of 1 mol of succinic acid was as follows:

\[
q_{cat} = (1 - \alpha) \Delta H_{cat}
\] (3)

According to this equation, \( \Delta H_{cat} \) was the enthalpy change corresponding to the catabolism of 1 mol of succinic acid.

By using the molecular growth yield reported in our earlier studies, i.e., 37.5 g/mol (3), the \( q_{an} \) value was found to be 32.5 \times 2.67 + 100.12 kJ/mol. This value was not negligible and contributed significantly to the experimental enthalpy change associated with the aerobic growth of \( E. coli \).

The quantity of heat that evolved during the growth, i.e., \( \Delta H_{net} \), was the sum of the enthalpy changes associated with the exergonic reactions \( (q_{cat}) \) and the enthalpy change associated with the synthetic reactions (i.e., \( q_{an} \)) according to the following equation:

\[
\Delta H_{net} = q_{cat} + q_{an} = (1 - \alpha) \Delta H_{cat} + \alpha \Delta H_{an}
\] (4)

In the case of the growth of \( E. coli \) on succinic acid, we showed in our earlier study that 41.5% of the succinic acid was recovered as cellular carbon (i.e., \( \alpha = 0.41 \)) and, consequently, that \( q_{cat} \) was found to be \(-876.32 \) kJ/mol. It therefore became easy to calculate the \( \Delta H_{cat} \) by using equation (4), as follows: \( \Delta H_{cat} = -876.32 + 100.12 = -776.2 \) kJ/mol. This last value is in excellent agreement with the experimental data already published (3), that is, \(-739.4 \) kJ/mol.

A second method could be applied to evaluate the enthalpy change associated with cell formation from succinic acid and a nitrogen source. The anabolism reactions could be scrutinized step by step and divided into the following three main parts. (i) First, the conversion of carbon and nitrogen source into the monomers of the macromolecules contained in 1 g of cells (dry weight). The sum of the enthalpy changes associated with the different reactions was identified as \( \Delta H_{an} \).

(ii) Second, the polymerization of these monomers into unfolded macromolecules. This second step was accompanied with an enthalpy change identified as \( \Delta H_{an} \).

(iii) Third, the enthalpy change associated with the folding of macromolecules was \( \Delta H_{an} \).

Therefore, the enthalpy change associated with the synthesis of 1 g of cells (dry weight) would be given by the following relationship:

\[
\Delta H_{an} = \Delta H_{an} + \Delta H_{an} + \Delta H_{an}
\] (5)

The calculation of \( \Delta H_{an} \) was listed, according to the stoichiometric relationship of monomer formation in Table 1. The results of this analysis showed that the synthesis of all amino acids contained in 1 g (dry weight) of cells was associated with an enthalpy decrease of \(+1.46 \) kJ/g. The contribution for the lipid content was of the same order of magnitude, that is, \(+1.29 \) kJ/g.

Polysaccharide synthesis, however, was accompanied by a negligible enthalpy increase, that is, \(+0.081 \) kJ/g. On the other hand, nucleic acid basis synthesis was slightly exothermic. This fact was probably due to the oxidation level of these compounds compared with succinic acid levels.

The change in enthalpy occurring during the formation of unfolded macromolecules (\( \Delta H_{an} \)) could be investigated from enthalpy changes associated with the hydrolysis reaction of the main bonds involved in macromolecules (peptide, glycosidic, acylester, and phosphodiester bonds). These data were taken from the literature (J. P. Belaich, in Biological Calorimetry, in press).

The \( \Delta H_{an} \) was endothermic and found to be \(+0.063 \) kJ/g (Table 2). The enthalpy change associated with the folding of macromolecules (\( \Delta H_{an} \)) could be estimated from the results of scanning calorimetry. Average values of \(-0.9 \), \(-14.33 \), and \(-36.15 \) kJ per monomer were found for the heat from the folding of protein, nucleic acid, and membrane bilayers, respectively (Belaich, in Biological Calorimetry, in press). So, with regard to these values the \( \Delta H_{an} \) was \(-0.017 \) kJ/g (dry weight) of cells.

Keeping these three \( \Delta H_{an} \) values in mind, and according to equation (5), we could predict the
Table 1. Reaction of synthesis from succinate and NH$_4$OH

<table>
<thead>
<tr>
<th>Monomer</th>
<th>Synthesis</th>
<th>$\Delta H_m$ (kJ/mol)</th>
<th>Amt of monomer per g of cell (Ah/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartate</td>
<td>C$_4$H$_6$O$_4$ + NH$_4$OH + 0.5 O$_2$ → C$_6$H$_5$N$_5$O$_2$ + 1.75 H$_2$O</td>
<td>-739.4 kJ/mol</td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>1.5 C$_6$H$_7$O$_6$ + 2 NH$_4$OH → C$_6$H$_7$O$_6$N$_2$ + 3.5 H$_2$O + 1.25 O$_2$</td>
<td>+437.85</td>
<td>403 +0.1764</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.25 C$_6$H$_7$O$_6$ + NH$_4$OH + H$_2$SO$_4$ → C$_6$H$_7$O$_6$NS + 1.75 H$_2$O + 3.125 O$_2$</td>
<td>+1,101.57</td>
<td>201 +0.2214</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>C$_6$H$_7$O$_6$ + NH$_4$OH → C$_6$H$_7$O$_6$N$_2$ + 0.5 O$_2$</td>
<td>+250.84</td>
<td>252 +0.0632</td>
</tr>
<tr>
<td>Proline</td>
<td>1.25 C$_6$H$_7$O$_6$ + NH$_4$OH → C$_6$H$_7$O$_6$N$_2$ + 1.75 H$_2$O + 0.125 O$_2$</td>
<td>+7.78</td>
<td>353 +0.0027</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.5 C$_6$H$_7$O$_6$ + 4 NH$_4$OH → C$_6$H$_7$O$_6$N$_4$ + 7.5 H$_2$O + 0.25 O$_2$</td>
<td>+494.64</td>
<td>252 +0.1246</td>
</tr>
<tr>
<td>Serine</td>
<td>0.75 C$_6$H$_7$O$_6$ + NH$_4$OH + 1.25 O$_2$ → C$_6$H$_7$O$_6$N$_2$ + 1.25 H$_2$O</td>
<td>-13</td>
<td>302 -0.0059</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.5 C$_6$H$_7$O$_6$ + NH$_4$OH + 0.5 O$_2$ → C$_6$H$_7$O$_6$N$_2$ + 1.75 H$_2$O</td>
<td>-115.45</td>
<td>403 +0.0465</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.75 C$_6$H$_7$O$_6$ + NH$_4$OH + H$_2$SO$_4$ → C$_6$H$_7$O$_6$NS + 2.25 H$_2$O + 1.975 O$_2$</td>
<td>+730.97</td>
<td>101 +0.0738</td>
</tr>
<tr>
<td><strong>Nucleic acid</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenine</td>
<td>1.25 C$_6$H$_7$O$_6$ + 5 NH$_4$OH + 1.875 O$_2$ → C$_6$H$_7$N$_5$O$_5$ + 13.75 H$_2$O</td>
<td>-810.98</td>
<td>140 -0.1135</td>
</tr>
<tr>
<td>Guanine</td>
<td>1.25 C$_6$H$_7$O$_6$ + 5 NH$_4$OH + 1.375 O$_2$ → C$_6$H$_7$O$_5$N$_3$ + 13.75 H$_2$O</td>
<td>-1,099.77</td>
<td>140 -0.1539</td>
</tr>
<tr>
<td>Cytosine</td>
<td>C$_6$H$_7$O$_6$ + 3 NH$_4$OH + 0.125 O$_2$ → C$_6$H$_7$N$_5$O$_5$ + 8 H$_2$O</td>
<td>-704.99</td>
<td>140 -0.0987</td>
</tr>
<tr>
<td>Uracil</td>
<td>C$_6$H$_7$O$_6$ + 2 NH$_4$OH + 0.25 O$_2$ → C$_6$H$_7$N$_3$O$_5$ + 6 H$_2$O</td>
<td>-492.99</td>
<td>115 -0.0567</td>
</tr>
<tr>
<td>Thymine</td>
<td>1.25 C$_6$H$_7$O$_6$ + 2 NH$_4$OH + 0.375 O$_2$ → C$_6$H$_7$N$_5$O$_5$ + 5.75 H$_2$O</td>
<td>-741.33</td>
<td>24 -0.0178</td>
</tr>
<tr>
<td><strong>Lipid</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>4 C$_6$H$_7$O$_6$ + 4 H$_2$O → C$_6$H$_4$O$_4$ + 9 O$_2$</td>
<td>+3,873.69</td>
<td>296 +1.1466</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0.75 C$_6$H$_7$O$_6$ + 1.75 H$_2$O → C$_6$H$_7$O$_6$N$_2$ + 0.875 O$_2$</td>
<td>+554.52</td>
<td>148 +0.0820</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>0.5 C$_6$H$_7$O$_6$ + NH$_4$OH → C$_6$H$_7$O$_6$N$_2$ + 0.5 H$_2$O + 0.75 O$_2$</td>
<td>+403.62</td>
<td>148 +0.0597</td>
</tr>
<tr>
<td><strong>Polysaccharide</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>14 C$_6$H$_12$O$_6$ + 1.5 H$_2$O → C$_6$H$_12$O$_6$ + 0.75 O$_2$</td>
<td>+135.85</td>
<td>602 +0.0817</td>
</tr>
</tbody>
</table>

* Value from Morowitz (5). The enthalpy of formation for all the molecules were calculated from Morowitz (5), except for succinic acid which was calculated as -940.16 kJ/mol (3).

Theoretical enthalpy change associated with the synthesis of 1 g of cells (dry weight) from succinic acid and ammonium hydroxide. This value was found to be slightly positive, +2.43 kJ/g of cells (dry weight). This value was in good agreement with the $\Delta h_m$ data calculated from the combustion enthalpy of succinic acid-grown cells (method 1), i.e., +2.67 kJ/g.

As was done in method 1, it was easy to calculate the heat quantity associated with the endergonic reactions during the metabolism of 1 mol of succinic acid with regard to these new data and according to equation (2). The $\phi_m$ was 37.5 × 2.43 = +91.12 kJ/mol.

The theoretical heat quantity evolved by bacteria growing on succinic acid as the energy and carbon source calculated by method 2 became $\Delta H_m = -876.32 + 91.11 = -785.2$ kJ/mol of succinic acid.

This second theoretical value was in good agreement with both that of method 1 and with the experimental data, i.e., -739.4 kJ/mol (3).

DISCUSSION

Until now, it has been commonly accepted that the heat from the combustion of microorganisms was similar to the heat from glucose combustion (1, 2, 4, 7; A. Granger, Ph.D. thesis, Aix-Marseille University, Marseille, France, 1963), and therefore it was assumed that enthalpy changes associated with the biosynthesis reactions were negligible (4; Belaich in Biologi-
Table 2. Enthalpy change associated with the three main steps in anabolism

<table>
<thead>
<tr>
<th>Nature of the polymer</th>
<th>Amt of compound* (μmol/g)</th>
<th>( \Delta H^\circ_{\text{monomer}} ) (kJ/g)</th>
<th>( \Delta H^\circ_{\text{monomer}} ) (kJ/g)</th>
<th>( \Delta H^\circ_{\text{monomer}} ) (kJ/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>4,785</td>
<td>+1.46</td>
<td>+8.36</td>
<td>+0.04</td>
</tr>
<tr>
<td>Nucleic acid</td>
<td>559</td>
<td>−0.44</td>
<td>+16.72</td>
<td>+0.009</td>
</tr>
<tr>
<td>Polysaccharide</td>
<td>602</td>
<td>+0.081</td>
<td>+12.54</td>
<td>+0.007</td>
</tr>
<tr>
<td>Lipid monomer</td>
<td>148</td>
<td>+1.29</td>
<td>+41.8</td>
<td>+0.006</td>
</tr>
<tr>
<td>Total enthalpy change</td>
<td></td>
<td>+2.39</td>
<td>+0.062</td>
<td>−0.017</td>
</tr>
</tbody>
</table>

* Values from Morowitz (5).

Values from Table 1.

Values from Belaich (in Biological Calorimetry, in press).

Thermodynamic analysis (Belaich Calorimetry). However, the recent measurements by Prochazka et al. did not agree with this assumption (6).

Using the Prochazka and Sedlaczeck values, Belaich (in Biological Calorimetry, in press) demonstrated that the discrepancy between these two methods strongly influenced values for \( \Delta H_{\text{an}} \), as a result, it became hazardous to predict based on data from thermodynamics literature the amount of heat evolved during microbial growth and even more hazardous to predict the amount of heat from the combustion of glucose. It is essential that one understand both the elementary analysis and the combustion of heat of bacterial cells to interpret accurately the thermochanical aspects of microbial growth.

Data reported in this paper showed that the heat combustion of E. coli cells (i.e., −22.9 kJ/g) was similar to the value published by Prochazka et al. (−23.11 kJ/g of cells [dry weight]).

These values are different from those of the heat from the combustion of glucose (i.e., 15.67 kJ/g).

The two investigational methods previously proposed (Belaich in Biological Calorimetry, in press) for evaluating the quantity of heat associated with 1 g of synthesized cells from succinic acid and ammonia gave similar values (\( \Delta H_{\text{an}} = +2.67 \) kJ/g and +2.43 kJ/g). As one would expect, both values of enthalpy change were positive and demonstrated that the anabolic processes were indeed endothermic.

The heat quantities associated with the biosynthetic reactions (\( q_{\text{an}} \)) during growth on 1 mol of succinic acid were easily calculated from the growth yield (\( Y \)) on succinic acid and both \( \Delta H_{\text{an}} \) values. These values were found to be +100.12 or +91.12 kJ/mol of succinic acid, which emphasized that the enthalpy change incorporated into cells during microbial growth could not be neglected. These values for \( q_{\text{an}} \) indicate the differences observed between the experimental data and those of the quantity of heat corresponding to the catabolic reaction corrected for the part of succinic acid incorporated into cellular carbon. This difference was found to be −136.96 kJ/mol in our earlier paper (3).

The enthalpy changes associated with the metabolism of a precise substrate were dependent on the amount of energy source incorporated into the cellular material and the enthalpy changes associated with both catabolic and anabolic reactions, respectively.

Equation (4) indicates that \( \Delta H_{\text{met}} \) was similar to \( \Delta H_{\text{an}} \) if values of \( Y \) and consequently \( \alpha \) were low. This was generally observed when cells were grown under anaerobic conditions in complex medium. Under those conditions, no significant differences were found between the experimental data and the calculated values corrected for the short part of the energy source incorporated. As a result, \( \Delta H_{\text{an}} \) was assumed to be negligible (Belaich, in Biological Calorimetry, in press). The calculation made by Belaich for this case showed that \( \Delta H_{\text{an}} \) was +0.39 kJ/g when a carbohydrate was the sole energy and carbon source.

In the case of bacterial growth performed under aerobic conditions, \( Y \) and \( \alpha \) were not negligible, so the calculation was done with regard to these parameters and the \( \Delta H_{\text{an}} \) value.

The coefficient could be determined for cells growing in minimal medium under a growth-limiting concentration of energy and carbon source according to the following equation:

\[
\alpha = \frac{y}{100} \cdot \frac{1}{Y} \cdot \frac{1}{12} \cdot \frac{1}{a}
\]

Here \( y \) was the carbon amount contained in 100 g of cells (dry weight) and could be calculated from the bacterial composition. \( Y \) is the molar growth yield; 12 is the atomic carbon weight, and \( a \) represents the number of carbon atoms in 1 mol of energy and carbon source.
In conclusion, it was possible to predict the quantity of heat associated with aerobic bacterial growth when the culture was grown under well-defined conditions in a minimal medium containing a growth-limiting concentration of carbon source. The contribution of anabolism strongly influenced the underlying heat evolution. The bacterial composition and the heat from the combustion of cells must be taken into account in thermochemical studies of bacterial growth.

LITERATURE CITED