

# THE FREE ENERGIES OF FORMATION OF AQUEOUS *d*-ALANINE, *l*-ASPARTIC ACID, AND *d*-GLUTAMIC ACID

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The employment of thermodynamics in biochemistry has been restricted, until recently, to the use of first law data. In the last few years a beginning has been made in the application of the second law; *i.e.*, of free energy data (1, 2). The development of this field is limited by the paucity of available free energy data. We have therefore undertaken the systematic determination of the free energies of formation of compounds which may be interesting in biochemistry or physiology.

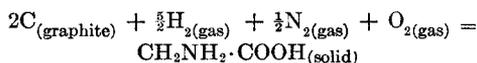
At the outset the query is met, of course, as to the universal validity of the second law of thermodynamics in biological systems. We shall deal with the general question in future publications. For the present it is profitable to assume that organisms obey the second law of thermodynamics as do macroscopic systems.

We shall assume, therefore, that the theoretical maximum amount of work derivable from any process is given by the equation<sup>1</sup>

$$w_R = -\Delta F + \Delta(PV) \quad (1)$$

where  $w_R$  is the work performed when the reaction is carried out in a perfectly reversible manner,  $-\Delta F$  the difference in free energy between initial and final states (*i.e.*  $F_1 - F_2$ ), and  $\Delta(PV)$  the change in the pressure-volume product,  $P_2V_2 - P_1V_1$ . Further in any spontaneous process  $-\Delta F$  is a positive quantity.

Let us consider the hypothetical reaction



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<sup>1</sup> The symbols used throughout are those of Lewis and Randall (3).

which is the equation for the formation of glycine from the elements. The free energy change for this reaction is given by the equation

$$\Delta F_{298} = \Delta H - T\Delta S \quad (2)$$

We can evaluate  $\Delta F$  if  $\Delta H$  and  $\Delta S$  are known at 25°.

$\Delta H_{298}$  may be obtained from the value of the heat of combustion at constant pressure and at 25°. It is simply the sum of the heat of combustion and the known heats of formation of the number of mols of water and carbon dioxide that are formed. This may be written in the form of the general equation

$$\Delta H = \text{heat of combustion} + a\Delta H_{\text{H}_2\text{O}} + b\Delta H_{\text{CO}_2} \quad (3)$$

where  $a$ ,  $b$ , etc., represent the number of mols of the substance taken.

In a like manner  $\Delta S_{298}$  is the difference between  $S_{298}$  of the compound and the corresponding known entropies of the elements. This may be written

$$\Delta S_{298} = S_{(\text{compound}, 298)} - aS_{(\text{C}, 298)} - bS_{(\text{H}_2, 298)} - cS_{(\text{O}_2, 298)} - dS_{(\text{N}_2, 298)} \quad (4)$$

If we accept the third law of thermodynamics, the entropy of the compound may be determined from the relation

$$S_{298} = \int_0^{298} \frac{C_p}{T} dT + \frac{\Delta H}{T} + \int_T^{298} \frac{C_p}{T} dT \quad (5)$$

This integral requires that the heat capacity and any heats of transition be known over the complete temperature range.

In our work we have determined  $C_p$  and any transitions from the temperature of liquid air to that of the room. Consequently we can evaluate the portion of the integral between 90–298°K. graphically. For the portion of the integral between 0–90°K. we have used the empirical extrapolation formula of Kelley, Parks, and Huffman (4). This method is based on actual experimental heat capacity measurements to very low temperatures.

The values we have used for the heats of formation of water and carbon dioxide and the values for the entropies of the elements are given in Table I.

It is obvious that the value of the free energy of formation of a

substance in its pure crystalline or liquid state will rarely be useful as such in physiological calculations. Most substances *in vivo* are in dilute solution, and as an additional complication, the hydrogen ion concentration varies with different experimental conditions, and from species to species. Further, at different times the substances under consideration are weak acids or bases, amphoteric electrolytes, or neutral compounds. In view of these considerations, the selection of one standard reference state for all compounds seems inadvisable. We shall, in preference, employ different standard states according to the substance and the case under consideration.

*d-Alanine*

Because of their bearing on several problems in nitrogen metabolism, we have begun with the determination of the free energies

TABLE I  
*Heats of Formation and Entropies of Elementary Data*

| Heats of formation        |                  |                   | Entropies of elements |           |                   |
|---------------------------|------------------|-------------------|-----------------------|-----------|-------------------|
| Substance                 | $\Delta H_{298}$ | Bibliographic No. | Element               | $S_{298}$ | Bibliographic No. |
| H <sub>2</sub> O (liquid) | -68,310          | 5                 | C (graphite)          | 1.3       | 7                 |
| CO <sub>2</sub> (gas)     | -94,240          | 6                 | H <sub>2</sub> (gas)  | 31.23     | 8                 |
|                           |                  |                   | N <sub>2</sub> (gas)  | 45.78     | 9                 |
|                           |                  |                   | O <sub>2</sub> (gas)  | 49.03     | 10                |

of *d*-alanine, *d*-glutamic acid, and *l*-aspartic acid. As an example of the way in which the heat capacity measurements are used in these calculations, experimental values for *d*-alanine are given in Table II. The combination of the graphical integration of these heat capacity data between 90–298°K. with the value determined by extrapolation from 90–0°K. gives the value for  $S_{298}$  of 31.6 E.U. This value combined with the  $S_{298}$  values for the elements (Table I) gives a value of -153.5 E.U. for the  $\Delta S_{298}$  of formation.

The heat of combustion of *d*-alanine at constant pressure and corrected to 25° is 387,200 calories. When this value is combined with the values for the heats of formation of water and carbon dioxide (Table I)  $\Delta H$  is -134,600 calories. If these values are substituted in Equation 2,  $\Delta F_{298} = -134,600 - (298.1)(-153.5) = -88,850$  calories.

In order to compute the free energy of a substance in solution

its solubility and corresponding activity coefficient must be known. By interpolation from the data given by Seidell (11), the solubility of *d*-alanine at 25° is 16.7 gm. in 100 gm. of water. According to Frankel (12) the activity coefficient of *d*-alanine in water at concentrations as high as 13.4 gm. in 100 gm. of water is 1. Freezing point depression and refractometric measurements at 30° gave the same result.

The equation for the concentration of the *Zwitter Ion* form is

$$\Sigma \text{ alanine} = A \left( 1 + \frac{(\text{H}^+)}{K_A} + \frac{K_W}{K_B \cdot (\text{H}^+)} \right) \quad (6)$$

The values for  $\text{p}K_A$  and  $(\text{p}K_W - \text{p}K_B)$  at 25° are 2.34 and 9.69 respectively (13). The hydrogen ion concentration of the satu-

TABLE II  
*Specific Heats at Constant Pressure of Crystalline d-Alanine*

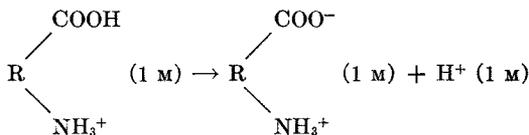
| Temperature | Specific heats at constant pressure $C_p$ | Temperature | Specific heats at constant pressure $C_p$ |
|-------------|---|-------------|---|
| °K.         | calories per gm.                          | °K.         | calories per gm.                          |
| 84.4        | 0.121                                     | 179.9       | 0.222                                     |
| 89.2        | 0.127                                     | 200.1       | 0.241                                     |
| 96.2        | 0.135                                     | 219.9       | 0.258                                     |
| 104.2       | 0.146                                     | 239.8       | 0.275                                     |
| 117.2       | 0.154                                     | 260.0       | 0.292                                     |
| 119.5       | 0.163                                     | 275.1       | 0.306                                     |
| 134.6       | 0.180                                     | 276.3       | 0.306                                     |
| 149.2       | 0.194                                     | 281.2       | 0.310                                     |
| 165.4       | 0.209                                     | 289.6       | 0.318                                     |
|             |   | 296.8       | 0.324                                     |

rated solution of alanine at 25° computed by means of the equation of Sørensen (14) is  $8.1 \times 10^{-7}$ . Insertion of these values in Equation 6 shows that all but a negligible quantity of the ampholyte is in the *Zwitter Ion* form.

The free energy change in the transfer of alanine from its saturated solution to a 1 M solution is

$$\begin{aligned} -\Delta F_{298} &= RT \ln 167/89.1 \\ &= (1.989)(298.1)(2.303) \log 167/89.1 = 370 \text{ calories} \\ \therefore \Delta F_{(\text{saturated solution})_{298}} - \Delta F_{(1 \text{ M})_{298}} &= 370 \text{ calories} \\ \therefore \Delta F_{(1 \text{ M})_{298}} &= -88,850 - 370 = -89,220 \text{ calories} \end{aligned}$$

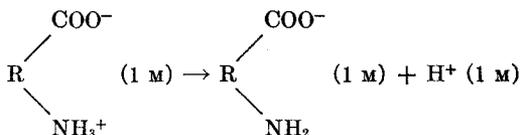
The free energies of the two ions may be computed as follows:



$$-\Delta F = RT \ln K_A = (-1365)(2.34) = -3190 \text{ calories}$$

$$\therefore \Delta F_{(\text{cation}, 1 \text{ M})_{298}} = -92,410 \text{ calories}$$

Similarly for the basic dissociation,



$$-\Delta F = RT \ln \frac{K_W}{K_B} = -1365 (\text{p}K_W - \text{p}K_B) = (-1365)(9.69)$$

$$= -13,230 \text{ calories}$$

$$\therefore \Delta F_{(\text{anion}, 1 \text{ M})_{298}} = -89,220 + 13,230 = -75,990 \text{ calories}$$

The constants employed in the calculation of the free energy changes in ionization are not strictly the thermodynamic dissociation constants. They are titration or apparent dissociation constants. These two types of constants are related to each other as follows:

$$K' = (\text{H}^+) \frac{(\text{A}^-)}{(\text{HA})} = \text{titration constant}$$

$$K = (\text{H}^+) \frac{(\text{A}^-)\gamma_-}{(\text{HA})\gamma_u} = \text{thermodynamic dissociation constant}$$

where  $\gamma_u$  denotes the activity coefficient of the undissociated acid.

On conversion to negative logarithms  $\text{p}K = \text{p}K' - \log \frac{\gamma_-}{\gamma_u}$   
 Simms (15) found that within the range of ionic strength from 0.05 to 0.3 the value of  $\text{p}K'$  for glycine was practically constant.

The activity coefficient ratio is therefore 1, and we may consider in the case of alanine as well as glycine  $\text{pK} = \text{pK}'$ .

*l*-Aspartic Acid

From values published elsewhere (16), the thermal data for *l*-aspartic acid are:

$$S_{298} = 41.5 \text{ E.U.}, \Delta S_{298} = -193.9 \text{ E.U.}$$

$$\Delta H_{298} = -231,300 \text{ calories}, \Delta F_{298} = -173,500 \text{ calories}$$

The solubility of *l*-aspartic acid at 25° is  $3.63 \times 10^{-2}$  mols in 1000 gm. of water (17). Because of the insufficiency of data regarding the activity coefficients of aspartic acid and its ions in the saturated solution, the computation of the free energy of solution can, at present, be only approximate. Two methods are open: One is the extrapolation of the calculations of Hoskins, Randall, and Schmidt (18) of the activity coefficients of aspartic acid to the saturated solution; the other is to calculate the degree of ionization of the aspartic acid in the saturated solution after computing the hydrogen ion concentration of the saturated solution at 25° by means of the equation of Sørensen (14), the titration constants at this temperature being employed. The value obtained for the concentration of the neutral aspartic acid by the second method may be taken, as an approximation, as equal to its activity (since titration constants and not the true dissociation constants were used in computing the pH of the saturated solution, and also the degree of dissociation). We have preferred the second method chiefly because of the uncertainty of the necessary double extrapolations of the activity coefficients to both a higher concentration and a higher temperature. The extrapolation of the activity coefficient from the freezing point to 25° is particularly uncertain because it is probable that with aspartic acid, on account of the great tendency of its neutral molecules to form aggregates, there is a considerable heat of dilution, which would make the activity coefficient at 25° quite different from that at the freezing point.

We have, nevertheless, computed the free energy of solution by both methods and have found the difference to be 50 calories. The details of only the second method are given. The values of the titration constants at 25° are  $\text{pK}_{A_1} = 1.90$ ;  $\text{pK}_{A_2} = 3.63$ ;

$(\text{p}K_W - \text{p}K_B) = 9.47$  (13). These yield a value for the hydrogen ion concentration in the saturated solution at  $25^\circ$  of  $5.5 \times 10^{-4}$ . The degree of ionization of aspartic acid is given by the equation

$$\Sigma \text{ aspartic acid} = A^\pm \left[ 1 + \frac{(\text{H}^+)}{K_{A_1}} + \frac{K_{A_2}}{(\text{H}^+)} + \frac{K_W \cdot K_{A_2}}{K_B \cdot (\text{H}^+)} \right] \quad (7)$$

When the above values are inserted in this equation,  $A^\pm = 0.72$ .

The free energy of solution at  $25^\circ$  is  $RT \ln (0.036) (0.72) = -2160$  calories.

The free energy of the neutral molecule at 1 M activity is  $\Delta F_{(A^\pm, 1 \text{ M})_{298}} = -173,500 + 2160 = -171,340$  calories.

The free energies of the ions of aspartic acid are, as in the case of alanine, as follows:

$$A^+ (1 \text{ M}) = A^\pm (1 \text{ M}) + \text{H}^+ (1 \text{ M})$$

$$-\Delta F = RT \ln K_{A_1} = -2590 \text{ calories}, \therefore \Delta F_{(A^+, 1 \text{ M})_{298}} = -173,930 \text{ calories}$$

$$A^\pm (1 \text{ M}) = A^\pm (1 \text{ M}) + \text{H}^+ (1 \text{ M})$$

$$-\Delta F = RT \ln K_{A_2} = -4950 \text{ calories}, \therefore \Delta F_{(A^\pm, 1 \text{ M})_{298}} = -166,390 \text{ calories}$$

$$A^\pm (1 \text{ M}) = A^- (1 \text{ M}) + \text{H}^+ (1 \text{ M})$$

$$-\Delta F = RT \ln \frac{K_W}{K_B} = -12,930 \text{ calories}, \therefore \Delta F_{(A^-, 1 \text{ M})_{298}} = -153,460 \text{ calories}$$

#### *d*-Glutamic Acid

The thermal data for *d*-glutamic acid are

$$S_{298} = 45.7 \text{ E.U.}, \Delta S_{298} = -222.3 \text{ E.U.}$$

$$\Delta H_{298} = -236,400 \text{ calories}, \Delta F_{298} = -170,200 \text{ calories}$$

The solubility at  $25^\circ$  is 0.058 mol in 1000 gm. of water (17). The free energy of solution was calculated by the method which was preferred and set out in detail for aspartic acid. The titration constants at  $25^\circ$  are  $\text{p}K_{A_1} = 2.10$ ,  $\text{p}K_{A_2} = 4.07$ , and  $(\text{p}K_W - \text{p}K_B) = 9.47$  (13). The hydrogen ion concentration of the saturated solution at  $25^\circ$  calculated by the method of Sørensen, is  $7.7 \times 10^{-4}$ . Insertion of these values into Equation 7 gives 83 per cent of the total glutamic acid in the neutral form in a saturated solution.

The free energy of solution therefore is  $RT \ln (0.83) (0.058) = -1800$  calories. The value obtained by extrapolating from the data of Hoskins, Randall, and Schmidt is  $-1890$  calories. If the first value is taken for the free energy of solution, the free energy of neutral glutamic acid at 1 M activity is  $-168,400$  calories. The free energies of the ions at  $25^\circ$  computed as in the case of aspartic acid, by the use of titration constants, are:

$$G^+ (1 \text{ M}) = G^\pm (1 \text{ M}) + H^+ (1 \text{ M})$$

$$-\Delta F = RT \ln K_{A_1} = -2870 \text{ calories}, \therefore \Delta F_{(G^+, 1 \text{ M})_{298}} = -171,270 \text{ calories}$$

$$G^\pm (1 \text{ M}) = G^\pm (1 \text{ M}) + H^+ (1 \text{ M})$$

$$-\Delta F = RT \ln K_{A_2} = -5560 \text{ calories}, \therefore \Delta F_{(G^\pm, 1 \text{ M})_{298}} = -162,840 \text{ calories}$$

$$G^\pm (1 \text{ M}) = G^\mp (1 \text{ M}) + H^+ (1 \text{ M})$$

$$-\Delta F = RT \ln \frac{K_W}{K_B} = -12,930 \text{ calories}, \therefore \Delta F_{(G^\mp, 1 \text{ M})_{298}} = -149,910 \text{ calories}$$

The rounded off values of the free energies of the different species of the three amino acids are collected in Table III.

#### *l-Aspartate-Enzyme-Fumarate-Ammonium Equilibrium*

Quastel and Woolf (19) observed that in the presence of *Bacillus coli communis* treated with growth inhibitors such as toluene, sodium nitrite, or propyl alcohol, an equilibrium is attained between *l*-aspartic acid, ammonia, and fumaric acid. Cook and Woolf (20) confirmed these results and extended the observations to other organisms. Woolf (21) found later that in the earlier formulation of the equilibrium they had overlooked the equilibrium between fumaric and *l*-malic acids. With this correction the value of the equilibrium constant  $\frac{(\text{fumaric acid})(\text{ammonia})}{(\textit{l}\text{-aspartic acid})}$  becomes approximately 0.01 at  $37^\circ$ .

With the determination of the free energy of *l*-aspartic acid, it became possible to compare this experimentally obtained equilibrium constant with that which can be computed from purely thermal data; *i.e.*, in which the intervention of the enzyme is ignored. Such a comparison of calculated and observed equilibrium constants was made in the case of the succinate-enzyme-

fumarate equilibrium. In that case it was found that the enzyme might be considered as a perfect catalyst (2).

On account of the complexity of the details in computing equilibria in solution from thermal data the following outline of the general plan is presented.

1. The equilibrium was measured at pH 7.4 and at 37°. It is necessary, therefore, to compute the free energies of the participants in the reaction at 37° from the given data at 25°.

(a) Reliable data regarding the heats of solution and of dilution of *l*-aspartic acid are not yet available. Accordingly, we have com-

TABLE III  
*Free Energies of Different Species of Three Amino Acids at 25°*

| Amino acid                   | Free energies of |                  |                                 |                                |                         |
|------------------------------|------------------|------------------|---------------------------------|--------------------------------|-------------------------|
|                              | Crystal-<br>line | Neutral<br>(1 M) | Mono-<br>valent<br>cation (1 M) | Mono-<br>valent<br>anion (1 M) | Divalent<br>anion (1 M) |
|                              | <i>calories</i>  | <i>calories</i>  | <i>calories</i>                 | <i>calories</i>                | <i>calories</i>         |
| <i>d</i> -Alanine.....       | -88,850          | -89,200          | -92,400                         | -76,000                        |                         |
| <i>l</i> -Aspartic acid..... | -173,500         | -171,300         | -173,900                        | -166,400                       | -153,450                |
| <i>d</i> -Glutamic ".....    | -170,200         | -168,400         | -171,250                        | -162,850                       | -149,900                |

puted the free energy of crystalline *l*-aspartic acid at 37° by means of the equation

$$\frac{\Delta F_2}{T_2} - \frac{\Delta F_1}{T_1} = \int_{T_1}^{T_2} - \frac{\Delta H}{T^2} dT \quad (8)$$

Over the short temperature range of 12°  $\Delta H$  may be taken as constant with a negligible error.

(b) Calculation of the free energy of solution at 37° from the solubility and degree of ionization at this temperature.

(c) Calculation of the free energy of the monovalent anion of *l*-aspartic acid from the free energy of the neutral molecule in solution and the dissociation constant at 37°.

(d, e) Similar calculations of the free energies of the bivalent fumarate ion and of the ammonium ion at 37°. At the pH at which the reaction is carried out we may consider all of the fumaric acid to be in the form of the bivalent ion, the ammonia as ammonium ion, and the *l*-aspartic acid as the monovalent anion.

2. Definition of the thermodynamic environment. (a) Computation of the ionic strength from the composition of the solution; (b) estimation of the activities of the various components of the reaction.

3. (a) Conversion of the given molal equilibrium concentrations into their corresponding activities with the corresponding modification of the value of the equilibrium constant.

(b) Calculation of the free energy change corresponding to this modified equilibrium constant by means of the equation  $-\Delta F = RT \ln K$ .

(c) Calculation of  $-\Delta F$  from the values in (1, c and d), derived from the thermal data, by means of the equation

$$-\Delta F(\text{reaction}) = \Delta F(\text{aspartate}^{\pm}, 1 \text{ M}) - \Delta F(\text{fumarate}^{\equiv}, 1 \text{ M}) \\ - \Delta F(\text{NH}_4^+, 1 \text{ M})$$

#### 1. (a) *l*-Aspartic Acid

$$\Delta F_{(\text{solid})_{298}} = -173,500, \Delta H_{298} = -231,300, \Delta F_{(\text{solid})_{310}} = -171,175 \text{ calories}$$

(b) The solubility of *l*-aspartic acid at 37° is  $5.5 \times 10^{-2}$  mols per 1000 gm. of water. From the data given by Cohn (13) the ionization constants at 37° are  $\text{pK}_{A_1} = 1.85$ ,  $\text{pK}_{A_2} = 3.57$ , and  $(\text{pK}_W - \text{pK}_B) = 9.12$ . The hydrogen ion concentration of the saturated solution is  $1.7 \times 10^{-3}$ . The fraction of the total aspartic acid in the neutral form according to the above values is 78 per cent. The free energy of solution therefore is  $RT \ln (0.055) (0.78) = -1940$  calories. The free energy of the neutral molecule at 1 M activity therefore is  $-171,175 + 1940 = -169,250$  calories.

(c) The free energy of the monovalent aspartate ion is given by the equation

$$A^{\pm} (1 \text{ M}) = A^{\pm} (1 \text{ M}) + \text{H}^+ (1 \text{ M})$$

$$-\Delta F = RT \ln K_{A_2} = -5050 \text{ calories}, \therefore \Delta F_{(A^{\pm}, 1 \text{ M})_{310}} = -164,200 \text{ calories}$$

(d) *Fumaric Acid*—We have previously calculated (2) the free energy of the bivalent fumarate ion at 1 M activity to be  $-144,630$  calories, and its heat of formation as  $-189,120$  calories. Hence the free energy of the bivalent fumarate ion at 37° is  $-142,850$  calories.

(e) *Ammonium Ion*—The free energy of  $\text{NH}_4^+$  at 298°K. is given by Lewis and Randall (3) as  $-18,930$  calories and the heat content as  $-31,790$ . Hence the free energy of this ion at 37° is  $-18,400$  calories.

2. (a) The ionic strength in one of the experiments quoted by Woolf was 0.22. (b) It is not possible from purely theoretical considerations to compute the precise activity coefficients of any of the participants in this reaction in solutions whose ionic strengths are as great as this. The available experimental data guide us only to a reasonable guess. From the following simplified form of the Debye-Hückel equation

$$-\log \gamma_i = \frac{0.5 z_i^2 \sqrt{\mu}}{1 + 3.3 \times 10^7 a \sqrt{\mu}} \quad (9)$$

where  $\gamma_i$  is the activity coefficient of the ions of the  $i$ th kind,  $z$  its valency,  $a$  is a constant taken as  $10^{-8}$ , and  $\mu$  the ionic strength, the activity coefficients of the ammonium ion, bivalent fumarate ion, and aspartate ion are 0.63, 0.15, and 0.63 respectively. The table given by Lewis and Randall ((3) p. 382) suggests that, if the ammonium ion resembles  $\text{Na}^+$ , or  $\text{K}^+$ , 0.7 is probably nearer the true value for its activity coefficient.

A value of the order of magnitude of 0.7 for the activity coefficient of the aspartate ion is similarly in accord with an extrapolation of the data of Hoskins, Randall, and Schmidt of the activity coefficient of monosodium aspartate. The measurements of Simms indicate that 3.3 instead of 4 is a more justifiable figure for  $z_i^2$  for the bivalent fumarate ion on account of the distance between the charges. This gives a value of 0.215 for the activity coefficient of this ion. At the pH of the experiment, approximately 7.35, 97 per cent of the ammonia is in the form of ammonium ion. In view of other uncertainties this correction may be neglected. All the fumaric acid may be taken as bivalent fumarate ion and the aspartic acid as the monovalent aspartate ion.

We are now in a position to attempt an approximate evaluation of the equilibrium constant. In the experiment which has been referred to above, the initial concentrations of fumaric acid and ammonia were 0.1 M. At equilibrium 28 per cent of the initial quantity of ammonia was found. The remainder had combined

with an equivalent amount of fumaric acid to form *l*-aspartic acid. The chemical reaction may be written  $l\text{-aspartate}^{\pm} = \text{fumarate}^{-} + \text{NH}_4^{+}$ .

The mass law expression, if activity coefficients are ignored, is  $\frac{(0.028)(0.028)}{(0.072)} = 0.011$ .

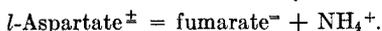
The free energy change is  $-\Delta F = RT \ln 0.011 = -2800$  calories.

When the activity coefficients are taken into account, the equilibrium expression is  $\frac{(0.028)(0.7)(0.028)(0.22)}{(0.072)(0.7)} = 0.0024$ .

The free energy change is  $-\Delta F = RT \ln 0.0024 = -3700$  calories.

TABLE IV

*Comparison of Free Energy Values Obtained from Thermal and Equilibrium Data for Reaction at 37°*



| Quantity  | Free energy values |
|---|--------------------|
|   | <i>calories</i>    |
| $\Delta F$ ( <i>l</i> -aspartate $^{\pm}$ )                                 | -166,000           |
|   | -164,200           |
|   | -163,500           |
| $\Delta F$ (fumarate $^{-}$ )   | -142,850           |
| $\Delta F$ (NH $_4^{+}$ )   | -18,400            |
| $-\Delta F$ (reaction) from thermal data                                    | -4,750             |
|   | -2,950             |
|   | -2,250             |
| $-\Delta F = RT \ln \frac{(C_1)(C_2)}{(C_3)}$                               | -2,800             |
| $-\Delta F = RT \ln \frac{(C_{1\gamma_1})(C_{2\gamma_2})}{(C_{3\gamma_3})}$ | -3,700             |

From the thermal data, by using the heat of combustion as given in the International Critical Tables (22) for aspartic acid, we find for the reaction  $-\Delta F_{310} = -164,200 + 142,850 + 18,400 = -2950$  calories.

From the values of Emery and Benedict (23) and of Fischer and Wrede (24) for the heat of combustion of *l*-aspartic acid two other values for  $-\Delta F$ , namely  $-4750$  and  $-2250$ , are obtained.

The observed value therefore agrees with that calculated within the limits of accuracy of the available thermal data. The con-

clusion is warranted, therefore, that here, as in the case of the succinate-fumarate equilibrium, the enzyme operates as a perfect catalyst. Stated in another form, we may conclude that the free energy values obtained from third law data may be used in the consideration of this reaction in metabolism, without taking into account the intervention of the enzyme.

The results of the above computations are collected in Table IV.

#### SUMMARY

1. The entropies, heats, and free energies of formation of *d*-alanine, *l*-aspartic acid, and *d*-glutamic acid, and their ions, at 25° are presented.

2. The free energy change in the reaction  $l\text{-aspartate}^{\pm} = \text{fumarate}^{\pm} + \text{NH}_4^+$  is computed from thermal data.

3. This value is shown to be in agreement with that calculated from the observed equilibrium effected by microorganisms, within the limits of accuracy of the thermal data.

4. It is concluded that the enzyme in this reaction operates as a perfect catalyst.

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