SUMMARY

The equilibrium constants of two transamination systems have been measured at 25°. The equilibrium constants, as defined in equations (1) and (2) were found to be, on the average, 6·74 in the glutamic-aspartic system and 1·52 in glutamic-alanine system.

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REFERENCES

Boulanger, P. & Osteux, R. (1949). *Bull. Soc. chim. Biol., Paris*, **31**, 1297.
Cohen, P. P. (1940). *J. biol. Chem.* **136**, 585.
Darling, S. (1945). *Acta physiol. scand.* **10**, 150.
Darling, S. (1947). *Nature, Lond.*, **160**, 403.
Krebs, H. A. (1948). *Biochem. J.* **43**, 51.
Krebs, H. A. (1950). *Biochem. J.* **47**, 605.
Krebs, H. A., Boulanger, P. & Osteux, R. (1950). *Bull. Soc. chim. Biol., Paris*, **32**, 851.
Krebs, H. A. & Johnson, W. A. (1937). *Biochem. J.* **31**, 645.
Lénárd, P. & Straub, F. B. (1942). *Stud. Inst. med. Chem. Univ. Szeged*, **2**, 59.
Meister, A., Sober, H. A. & Tice, S. V. (1951). *J. biol. Chem.* **189**, 577.
O'Kane, D. E. & Gunsalus, I. C. (1947). *J. biol. Chem.* **170**, 425.
Ostern, P. (1933). *Hoppe Seyl. Z.* **218**, 166.

The Free-energy Changes for the Reduction of Diphosphopyridine Nucleotide and the Dehydrogenation of L-Malate and L-Glycerol 1-Phosphate

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The free energy of reduction of diphosphopyridine nucleotide (DPN) may be evaluated by adding the free-energy change calculated from the equilibrium of a suitable DPN dehydrogenase system (1) to the free-energy change for the reduction of the substrate (2)

$$AH_2 + DPN^+ \rightarrow A + DPNH + H^+, \quad (1)$$

$$A + H_2 \rightarrow AH_2, \quad (2)$$

$$DPN^+ + H_2 \rightarrow DPNH + H^+. \quad (3)$$

The standard free-energy change (ΔG^0) of reaction (3) is related to the oxidation-reduction potential (E_0')

$$E_0' = -\frac{\Delta G^0}{2F} - \frac{RT\,\mathrm{pH}}{2F \log e},$$

where F is the Faraday (23068 cal.), R the gas constant and T the absolute temperature. By this procedure, several workers have obtained values for the oxidation-reduction potential of DPN using data for different hydrogenase systems (Green & Dewan, 1937; Clark, 1938; Schlenk, Hellström & Euler, 1938; Borsook, 1940). Several serious discrepancies appear when the available free-energy data for different dehydrogenase systems are critically examined, e.g. the generally accepted value for the DPN potential (−0·282 V. at pH 7 and 30°; Borsook, 1940) differs from that (−0·320 V. at pH 7 and 25°) which may be obtained from equilibrium and thermochemical data for the ethanol-DPN-acetaldehyde system (Dixon, 1949; Burton, 1952). A value closer to that of Borsook (namely −0·29 V. at pH 7 and 25°) is indicated by the potential measurements of Barron & Hastings (1934) and the equilibrium data of Kubowitz & Ott (1943) and Racker (1950) for the lactic dehydrogenase system. Another value of about −0·21 V. may be obtained by combining the potential measurements of Lehmann & Hoff-Jörgensen (1939) for the malate-oxaloacetate reaction with equilibrium data given by Schlenk *et al.* (1938).

The object of the work described in this paper was, first, to obtain a reliable independent value for the free-energy change (ΔG^0) for the reduction of DPN (i.e. the oxidation-reduction potential), and secondly to use this value in conjunction with equilibrium data to obtain the free-energy change of several biological dehydrogenations. To evaluate ΔG^0 for the reduction of DPN, the equilibrium constant has been measured for the reaction:

*iso*propanol + DPN^+ → acetone + DPNH + H^+, (4)

which is catalysed by crystalline preparations of yeast alcohol dehydrogenase. This reaction was

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investigated because accurate free-energy data are available for both acetone and *iso*propanol from thermochemical measurements. The value obtained for ΔG^0 of reduction of DPN from these data is in good agreement with that similarly obtained using published equilibrium and thermochemical data for the ethanol-acetaldehyde system. Equilibrium measurements are also reported for the malic and glycerol 1-phosphate dehydrogenase systems.

As will be shown in a later paper (Burton & Krebs, 1953), the data obtained here may be combined with other data to give comprehensive free-energy data for glycolysis and the citric acid cycle.

The value for the oxidation-reduction potential of DPN has been briefly reported elsewhere (Burton, 1952).

THERMODYNAMIC SYMBOLS AND CONVENTIONS

The conventions adopted are those of Lewis & Randall (1923) and the symbols are defined in the following paper with the exception of E'_0. This is the equilibrium e.m.f. of a cell consisting of an inert electrode in the aqueous oxidation-reduction system at a given pH and a hydrogen electrode (hydrogen at 1 atm.) in the same solution: all relevant components except acid are at unit activity. An account of the theory of oxidation-reduction potentials is given by Johnson (1949).

The unit of energy used in the text is the kilocalorie (kcal.), but some of the more important values (Table 3) are given both in kcal. and in kilojoules (kJ.). 1 calorie = 4·1840 absolute joules.

EXPERIMENTAL AND RESULTS

Diphosphopyridine nucleotide (DPN). This was obtained from local brewer's yeast by a method similar to that of LePage (1949), developed in co-operation with Mr D. E. Hughes. The stages after preparation of the crude extracts were performed in a different order, namely (*a*) charcoal adsorption and elution, (*b*) treatment with lead acetate, (*c*) precipitation and subsequent decomposition of the silver salt, and (*d*) precipitation by acidified acetone. The final products of several preparations were 70–85% pure. Yield 0·05 g./kg. yeast. The material used for the experiments described in this paper was 74% pure (calculated on the basis of the molecular formula $C_{21}H_{27}O_{14}N_7P_2$, mol.wt. 663). DPN was estimated spectrophotometrically after enzymic reduction by ethanol (Racker, 1950); the molecular extinction coefficient of reduced DPN at 340 mμ. was taken as $6{\cdot}22 \times 10^3$ cm.^{2}/mole (Horecker & Kornberg, 1948).

Enzymes. Highly purified preparations of malic dehydrogenase (Straub, 1942) and diaphorase (Straub, 1939) were from horse heart muscle; crystalline alcohol dehydrogenase (Racker, 1950) was from 'Premier' baker's yeast. A crude preparation of glycerol 1-phosphate dehydrogenase was obtained by fractionating an aqueous extract of guinea-pig skeletal muscle between 30 and 55% saturation with $(NH_4)_2SO_4$ (Racker, 1947). Before use all enzyme preparations were dialysed with stirring against 0·05 M-phosphate buffer, pH 7·4, to remove $(NH_4)_2SO_4$.

*iso*Propanol. This was purified according to Gilson (1932).

pH. This was measured at 25° using a glass electrode, 3·5 M-KCl bridge and 3·5 N-calomel electrode. The system was calibrated according to British Standard 1647: 1950.

Spectrophotometric measurements. These were made with a Beckman model DU spectrophotometer fitted with a constant temperature chamber (cf. Gibson & Balcom, 1947).

Enzymic dehydrogenation of isopropanol

The decolorization of methylene blue in the presence of *iso*propanol and a crude preparation of yeast alcohol dehydrogenase was described by Müller (1934), but the product of the reaction was not identified and the requirement for coenzyme not examined.

The reaction has been further investigated using crystalline yeast alcohol dehydrogenase. In the presence of excess *iso*propanol DPN was completely reduced as indicated by the light absorption at 340 mμ. On subsequent addition of acetone the reduced DPN could be reoxidized. These observations suggested that yeast alcohol dehydrogenase can catalyse reaction (4) in addition to the analogous reaction between ethanol and acetaldehyde. About 500 times as much enzyme is required to obtain the same rate of reaction with 0·03 M-*iso*propanol as that obtained with the same concentration of ethanol (pH 9; 8×10^{-5} M-DPN).

To confirm that acetone is the product of the reaction, it was isolated as its 2:4-dinitrophenylhydrazine derivative. A solution containing alcohol dehydrogenase (9×10^6 units; Racker, 1950), 70 mg. *iso*propanol, 8 mg. methylene blue, diaphorase (equivalent to 3×10^{-11} moles riboflavin) and 40 mg. 50% pure DPN in a total volume of 20 ml. was oxygenated by shaking in a 100 ml. reaction vessel fitted to a Warburg manometer (Krebs & Eggleston, 1945). After 6 hr., the rate of O_2 consumption became very slow. On the assumption that the O_2 was reduced to H_2O_2, the amount consumed (3·8 ml.) corresponded to 15% conversion of *iso*propanol to acetone. The enzymes were then inactivated by adding 0·8 ml. 2 N-NaOH and the solution was distilled for 15 min. into a receiving flask containing 10 ml. 2 N-HCl saturated with 2:4-dinitrophenylhydrazine. A copious yellow crystalline precipitate was immediately formed. No precipitate was formed in a control experiment where the *iso*propanol was added after inactivation of the enzymes by alkali. The material was twice recrystallized from aqueous ethanol and identified as acetone 2:4-dinitrophenylhydrazone by its m.p. (126° corr.) and the mixed m.p. (125°) with an authentic sample which itself melted at 125°. Values for the m.p. of this compound given in the literature are 122–124° (Dirscherl & Nahm, 1940) and 125–126·5° (Roberts & Green, 1946); it is not stated whether these values were corrected for the emergent stem.

Although it is feasible that reaction (4) is catalysed not by the enzyme which acts on ethanol but by an impurity in the enzyme preparation, this is considered to be unlikely since the ratio of the rates towards *iso*propanol and ethanol were similar at different stages of purification.

Equilibrium measurements. Most measurements were performed in pairs, each with the same concentration of constituents but added in different orders so that the equilibrium was approached from opposite sides (Fig. 1).

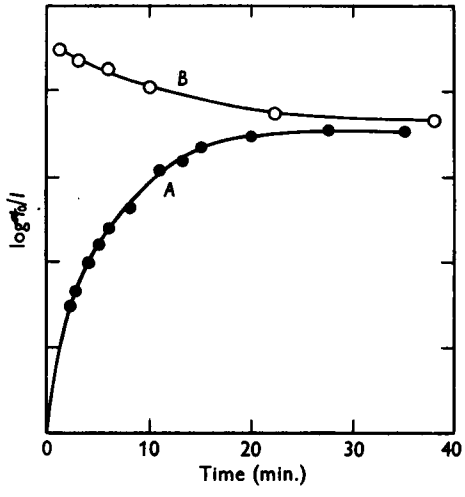

Fig. 1. Equilibrium of the *iso*propanol-DPN-acetone reaction approached from both sides. See Table 1. Temp., 25°; pH 7·64; $5·5 \times 10^{-3}$ M-pyrophosphate; 0·0195 M-acetone; 0·0978 M-*iso*propanol; 10^{-4} M-DPN.

In the first measurement of each pair (*A*) crystalline alcohol dehydrogenase was added to a solution containing acetone, *iso*propanol, and DPN. The reduction of the DPN was followed spectrophotometrically and allowed to proceed to equilibrium. In the second measurement (*B*), the enzyme was added to the same solution without acetone. The reduction of DPN by *iso*propanol was allowed to proceed beyond the equilibrium value obtained in (*A*). Acetone was then added and the reduced DPN became reoxidized until equilibrium was attained.

The equilibrium concentrations of acetone and *iso*propanol were taken as the initial values since the proportion reacting was in each case less than about 0·2%. Although values may be obtained for the concentrations of reduced and oxidized DPN at equilibrium from the initial concentrations and the spectrophotometer readings, the following procedure was adopted to ensure that no side reaction, such as destruction of the nucleotide, had occurred to any appreciable extent. The equilibrium concentration of reduced DPN was determined by adding 0·01 ml. of 10% acetaldehyde to 1·5 ml. of the equilibrium mixture. The optical density rapidly decreased to a value very close to that of the original solution of DPN, buffer, *iso*propanol and acetone. This fall in optical density was used to calculate the reduced DPN present at equilibrium. The oxidized DPN was determined from the increase of optical density when 2·0 ml. of the equilibrium mixture was mixed with 0·5 ml. of a solution containing 0·25 M-$Na_4P_2O_7$ and 0·5 M-ethanol. Under these conditions all the DPN present became reduced by the action of the ethanol and the dehydrogenase.

It will be seen from Table 1 that within the range investigated the equilibrium constant (K_c) is independent of the pH and the concentrations of the reactants. The mean of the twenty-six values found for K_c is $7·19 \times 10^{-9}$ M (range 5·89–8·91). In these measurements the total ionic strengths were very low (approx. 0·04) so that the activity coefficients of all the reactants may be taken as unity. Hence, the thermodynamic equilibrium constant (K_a), which is based on activities rather than concentrations, has in this case the same value as the concentration constant K_c. The ΔG^0 value calculated from the mean value for K_c is 11·11 kcal. and the range of ΔG^0 corresponding to the individual values of K_c is 10·98–11·23 kcal.

The protein concentrations given in Table 1 were obtained from the enzyme activity and Negelein & Wulff's (1937) data for the activity of their purified preparations per mg. protein. This procedure is valid since the purity (activity/optical density at 280 mμ.) of the alcohol dehydrogenase used in the experiments reported here was approximately the same as that of Negelein & Wulff's preparation. Theorell & Bonnischen (1951) have found an apparent effect of high enzyme concentration on the equilibrium of the liver alcohol dehydrogenase system. This may be explained by the assumption that the enzyme combines with reduced in preference to oxidized DPN, so that the total amount of reduced DPN at equilibrium is increased in the presence of high concentrations of enzyme. It will be seen that a 30-fold increase in the enzyme concentration did not appreciably affect the values obtained for K_c in the equilibrium measurements reported in Table 1.

The crystalline yeast alcohol dehydrogenase appears to have been contaminated with small amounts of an enzyme which catalyses the destruction of DPN. At the highest enzyme concentrations 20–40% destruction was observed if the incubation period was prolonged for 30 min. after equilibrium had been attained. It has previously been reported that crystalline enzyme preparations (e.g. ribonuclease, McDonald, 1949) may contain small amounts of other enzymes present as impurities.

Malic dehydrogenase system

L-Malate + DPN^+ → oxaloacetate + DPNH + H^+

The equilibrium of this system was measured by adding malic dehydrogenase (1·5–3 μg.) to a solution (3·7 ml.) containing L-malate, DPN and $2·7 \times 10^{-2}$ M-glycine or pyrophosphate buffer. The optical density at 340 mμ. rose rapidly and became almost constant after 3–5 min., the subsequent increase in the next 30 min. being only 3–5%. This slow change was almost certainly due to the spontaneous decomposition of oxaloacetate formed in the reaction. The equilibrium concentration of reduced DPN was obtained from the increase of optical density in the first 10 min. and that of oxaloacetate was assumed to be the same. The concentrations of L-malate and DPN were obtained from the initial concentrations and those of the reduced DPN formed.

Table 1. *Equilibrium in the presence of* isopropanol, acetone, *DPN and alcohol dehydrogenase*

(Temp., 25°; $5{\cdot}5 \times 10^{-3}$ M-pyrophosphate buffer. Equilibrium was approached (*A*) from excess of DPN^+ or, (*B*) from excess of DPNH (see text). Concentrations are final values.)

$$K_c = \frac{[\text{acetone}] \times [\text{DPNH}]}{[\textit{iso}\text{propanol}] \times [\text{DPN}^+]} \times \frac{1}{\text{antilog pH}}.$$

Protein concentration (μg./ml.)	pH	Acetone (M $\times 10^2$)	*iso*Propanol (M $\times 10^2$)	DPNH (M $\times 10^5$)	DPN^+ (M $\times 10^5$)	K_c (M $\times 10^9$)	
27	8·78	19·5	3·92	4·74	5·41	7·23	*A*
27	8·83	19·5	3·92	5·07	5·36	6·95	*B*
27	8·82	29·6	3·90	5·94	8·47	8·05	*A*
27	8·81	29·6	3·90	6·16	8·12	8·91	*B*
54	8·82	19·6	5·87	6·16	3·48	8·90	*B*
54	7·51	3·82	7·80	3·33	7·30	6·88	*A*
54	7·51	3·82	7·80	3·48	7·15	7·36	*B*
54	7·56	1·93	7·80	5·36	4·95	7·45	*A*
54	7·52	1·93	7·80	4·92	4·63	7·95	*A*
54	7·52	1·93	7·80	4·86	4·26	8·55	*B*
38	7·68	1·95	3·93	3·70	5·60	6·85	*A*
38	7·68	1·95	3·93	3·72	5·60	6·86	*B*
22	7·64	1·94	9·78	5·75	3·92	6·66	*A*
22	7·64	1·94	9·78	5·94	3·83	7·05	*B*
43	7·63	1·94	7·81	4·97	4·91	5·90	*A*
43	7·63	1·94	7·81	5·18	4·77	6·34	*B*
43	7·52	3·82	3·92	3·58	15·5	6·80	*A*
43	7·52	3·82	3·92	3·64	15·4	6·95	*B*
43	7·68	3·83	3·93	2·65	7·16	7·52	*A*
43	7·68	3·83	3·93	2·57	7·43	6·99	*B*
36	7·28	1·54	8·49	4·51	6·00	7·16	*A*
72	7·28	1·54	8·49	4·58	6·10	7·16	*A*
290	7·28	1·53	8·41	4·35	5·90	7·02	*A*
290	7·30	1·53	8·41	4·09	5·79	6·45	*A*
720	7·03	1·51	8·33	2·85	6·82	5·89	*A*
720	7·18	1·51	8·33	3·43	5·72	7·20	*A*
					Mean	7·19	

Table 2. *Malic dehydrogenase equilibrium*

(Temp., 25°. The first value is the mean of twelve similar measurements which gave values of K_c between $6{\cdot}85 \times 10^{-13}$ and $8{\cdot}75 \times 10^{-13}$ M. Glycine buffer ($2{\cdot}7 \times 10^{-3}$ M) was used in all except the last four measurements where pyrophosphate was used. Concentrations are final values.)

$$K_c = \frac{[\text{DPNH}]\,[\text{oxaloacetate}]}{[\text{DPN}^+]\,[\text{L-malate}]} \times \frac{1}{\text{antilog pH}}.$$

pH	DPNH (=oxaloacetate) (M $\times 10^5$)	DPN^+ (M $\times 10^5$)	L-Malate (M $\times 10^3$)	K_c (M $\times 10^{13}$)
8·83	1·45	7·50	5·28	7·87
8·87	1·77	11·8	5·01	7·15
8·95	2·06	11·6	5·28	7·76
8·63	1·77	16·5	5·28	8·41
8·85	2·32	16·0	5·28	9·00
8·87	2·06	16·0	5·13	7·00
8·89	2·42	21·2	5·28	6·75
8·86	2·42	21·2	5·28	7·22
8·81	2·82	32·4	5·27	7·23
8·78	2·86	35·7	5·27	7·22
8·75	2·90	35·8	5·27	7·96
8·83	3·27	41·3	5·14	7·46
8·19	1·53	42·7	5·17	6·86
8·08	1·35	43·1	5·17	6·79
7·55	0·79	43·7	5·19	7·76
			Mean	7·50

No destruction of DPN was detected in this system. As will be seen from Table 2, K_c is practically independent of pH or the concentration of DPN. It was approximately doubled when the concentration of L-malate was increased from about 5×10^{-3} to 5×10^{-2} M. Addition of sodium chloride or sodium fumarate to a reaction system containing 5×10^{-3} M-L-malate also increased K_c. Fumarase was absent so that the fumarate did not cause an apparent increase in K_c by being converted to malate. The increase of K_c accompanying the increased ionic strength can be satisfactorily explained by its effect on the activity coefficients of the reactants. According to the Debye-Hückel limiting law, at very low ionic strengths,

$$\log \gamma = -0{\cdot}5\, z^2 \sqrt{I},$$

where γ is the activity coefficient of an ion of valency z at an ionic strength I. Measurements of Hoskins, Randall & Schmidt (1930), indicate that for the dipolar aspartate^{+2-} and glutamate^{+2-} ions, the limiting law is approximately

$$\log \gamma = -1{\cdot}3 \sqrt{I}.$$

Assuming that the DPN^{+2-} ion also obeys this relation, and applying the limiting law to evaluate the activity coefficients of the other ions in the malic dehydrogenase system, we may obtain for very low ionic strengths,

$$\log K_c = \log K_a + 0{\cdot}7 \sqrt{I},$$

where K_a is the thermodynamic equilibrium constant.

The experimental results are shown in Fig. 2, where $(13 + \log K_c)$ has been plotted against $\sqrt{I}$. Neglecting the two values at the highest ionic strength (0·35), statistical analysis gives a regression line with a slope of 0·770 (S.D. 0·096) and an intercept of 0·777 (S.D. 0·069) at $\sqrt{I} = 0$. The value of the intercept corresponds to $K_a = 6{\cdot}0 \times 10^{-13}$ M and to $\Delta G^0 = 16{\cdot}67$ kcal. (S.D. 0·094 kcal.).

The values obtained at the higher ionic strengths are in good agreement with those of Stern, Ochoa & Lynen (1952) and are within the range of the values given by Schlenk *et al.* (1938).

Reaction with triphosphopyridine nucleotide (TPN). Under the influence of malic dehydrogenase, reduced TPN reacts with oxaloacetate at about 0·03 times the rate of reaction for reduced DPN (Mehler, Kornberg, Grisolia & Ochoa, 1948). An attempt was made to measure the equilibrium constant with TPN for comparison with that obtained using DPN. This was not successful because the rate of reaction between L-malate and TPN was so slow that equilibrium was not attained during the experimental period of 10 hr. The ratio of the initial rates of reaction with DPN and TPN was found to be approximately 6×10^4:1 starting with 0·22 M-L-malate and $1{\cdot}2 \times 10^{-4}$ M-DPN or TPN at pH 9·2; in the reverse direction it was 40:1 (pH 6·9; 8×10^{-5} M-oxaloacetate; 4×10^{-5} M reduced DPN or TPN). The latter ratio agrees with the results of Mehler *et al.* (1948).

Olson & Anfinsen (1953) have found that the equilibrium constant with DPN in the glutamic dehydrogenase system is fairly close to that with TPN. Their results give $K_c = 1{\cdot}45 \times 10^{-13}$ with DPN and $0{\cdot}99 \times 10^{-13}$ with the TPN. The ratio of the two constants are expected to be similar in the malic dehydrogenase system.

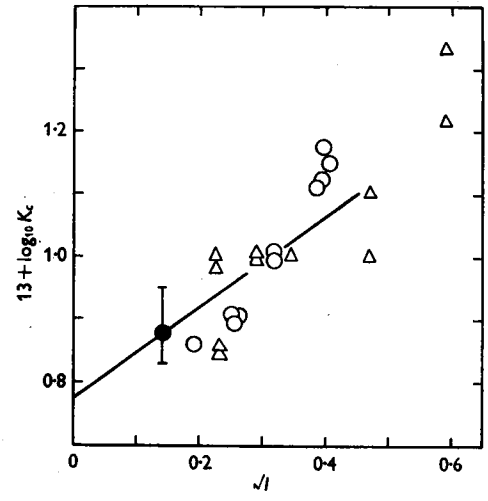

Fig. 2. Effect of ionic strength (I) on the equilibrium constant (K_c) of the malic dehydrogenase system. Temp., 25°; glycine buffer, $2{\cdot}7 \times 10^{-3}$ M; pH, 8·7–8·9; total concentration of DPN, 9×10^{-5} M. ●, range and mean of values shown in Table 1 with approximately 5×10^{-3} M-disodium L-malate: ○, values obtained with higher concentrations of L-malate; △, values with 5×10^{-3} M-L-malate and added NaCl. The equation of the regression line has been calculated omitting the two points at the highest ionic strength.

L-Glycerol 1-phosphate dehydrogenase system

The equilibrium of this system has previously been measured by Euler, Adler & Günther (1937) and by Baranowski (1949). In the following paper (Burton & Krebs, 1953) it will be shown that the equilibrium constant of this system may be used as part of a calculation of ΔG^0 for the hydrolysis of the terminal phosphate groups of adenosinetriphosphate. In view of the importance of a reliable ΔG^0 value for this reaction, and as the data of Euler *et al.* (1937) and of Baranowski are not very consistent, further equilibrium measurements have been made on the glycerol 1-phosphate dehydrogenase system. The product of the dehydrogenation is dihydroxyacetone phosphate (DHAP):

$$\text{L-Glycerol 1-phosphate} + \text{DPN}^+ \rightleftharpoons \text{DHAP} + \text{DPNH} + \text{H}^+. \quad (5)$$

Isomerase and aldolase were also present in the enzyme preparation used so that glyceraldehyde 3-phosphate (GAP) and fructose 1:6-diphosphate (HDP) are also formed:

$$\text{DHAP} \rightleftharpoons \text{GAP}, \quad (6)$$

$$\text{DHAP} + \text{GAP} \rightleftharpoons \text{HDP}. \quad (7)$$

The equilibria of reactions (6) and (7) are such that, at the concentrations attained, dihydroxyacetone phosphate represents about 90 % of the equilibrium mixture.

Equilibrium measurements. The enzyme preparation was added to a solution containing DPN and DL-glycerol 1-phosphate. The final protein concentration was approximately 0·3 μg./ml.; at this concentration the light absorption at 340 mμ. was negligible. In the first 1–2 min. after adding the enzyme, the optical density at 340 mμ. increased rapidly and was then constant at the new level for at least 40 min. No destruction of DPN was detected. The equilibrium concentration of reduced DPN was obtained from the increase of optical density after addition of the enzyme; the concentrations of DPN and L-glycerol 1-phosphate were obtained by difference from those initially present. The concentration of dihydroxyacetone phosphate was obtained from that of reduced DPN by subtracting a correction for the GAP and HDP which were formed by reactions (6) and (7):

$$[\text{DHAP}] = [\text{DPNH}] - [\text{GAP}] - 2\,[\text{HDP}]. \quad (8)$$

These corrections were evaluated using the equilibrium constants of reactions (6) and (7) which have been obtained from the data of Meyerhof & Junowicz-Kocholaty (1943); the value for reaction (7) has been extrapolated to 25° from their data at 30–60°:

$$[\text{DHAP}]/[\text{GAP}] = 22,$$
$$[\text{DHAP}] \times [\text{GAP}]/[\text{HDP}] = 6{\cdot}0 \times 10^{-5}.$$

Even if there are errors of as much as 50 % in each of these values, only a negligible error (4 %) would be introduced into the value obtained for the equilibrium constant of reaction (5). A further correction must be considered for the reaction between triosephosphate and DPN catalysed by the triosephosphate dehydrogenase. However, calculations from the equilibrium data of Meyerhof & Oesper (1947) show that this correction is negligible (<1 %).

The pH was varied between 7·5 and 9·2 using both veronal (diethyl barbituric acid) and pyrophosphate (3×10^{-3} M) buffers. The concentration of L-glycerol 1-phosphate was varied between 10^{-3} and 10^{-2} M, the total concentration of DPN between $1{\cdot}7 \times 10^{-5}$ M and 10^{-4} M and the concentration of reduced DPN between 3 and 5×10^{-5} M.

Sixteen values were obtained for K_c at 25°:

$$K_c = \frac{[\text{DPNH}]\,[\text{dihydroxyacetone phosphate}]}{[\text{DPN}^+]\,[\text{L-glycerol 1-phosphate}]} \times \frac{1}{\text{antilog pH}}.$$

The range of values for K_c is $4{\cdot}81$–$6{\cdot}51 \times 10^{-12}$ M with a mean of $5{\cdot}5 \times 10^{-12}$ M. The ionic strength in most of these experiments was about 0·03. By comparison with the effect of ionic strength on the malic dehydrogenase system (Fig. 2), extrapolation to zero ionic strength gives $K_a = 4{\cdot}6 \times 10^{-12}$ M. These measurements are in good agreement with that of Baranowski (1949) who used a crystalline enzyme preparation free from aldolase, isomerase and triosephosphate dehydrogenase. His data give $K_c = 7 \times 10^{-12}$ M at 22° and at an ionic strength of about 0·15. Euler *et al.* (1937) reported three measurements with a crude enzyme preparation: these give $K_c = 1{\cdot}4$, $5{\cdot}1$ and 5×10^{-12} M respectively, after correction for the glyceraldehyde phosphate and HDP formed and also using the value of Horecker & Kornberg (1948) for the molecular extinction coefficient of reduced DPN.

DISCUSSION

ΔG^0 for the reduction of DPN. This may be obtained from the equilibria in the presence of alcohol dehydrogenase and either (*a*) ethanol and acetaldehyde, or (*b*) *iso*propanol and acetone, together with the free-energy data which are available for these reactants.

As previously reported (Burton, 1952), the thermochemical data for aqueous ethanol and acetaldehyde give $\Delta G^0 = -9{\cdot}68$ kcal. for the reaction

$$\text{CH}_3\text{CHO (aq.)} = \text{CH}_3\text{CH}_2\text{OH (aq.)} + \text{H}_2\,(g). \quad (9)$$

The error in this value may be taken to be less than 0·4 kcal. The measurements of Racker (1950) give $K_c = 1{\cdot}15 \times 10^{-11}$ for the equilibrium with DPN in the presence of crystalline alcohol dehydrogenase at 25° and low ionic strengths (0·04). Assuming the activity coefficients of all the reactants to be unity, $\Delta G^0 = 14{\cdot}94$ kcal. Adding this value to ΔG^0 for reaction (9)

$$\text{DPN}^+ + \text{H}_2 = \text{DPNH} + \text{H}^+;\ \Delta G^0 = 5{\cdot}26 \text{ kcal.} \quad (10)$$

Racker's equilibrium measurements are in good agreement with those made at 20° and higher ionic strengths by Negelein & Wulff (1937) and by Theorell & Bonnischen (1951).

The reliable combustion and entropy data for liquid acetone and *iso*propanol (Miles & Hunt, 1941; Parks, Moseley & Peterson, 1950; Kelley, 1929) give $\Delta G^0 f$ values of $-37{\cdot}18$ and $-43{\cdot}26$ kcal. respectively. By combining these values with those for the vapour pressures (Beare, McVicar & Ferguson, 1930; Butler, Ramchandani & Thomson, 1935) $\Delta G^0 f$ is found to be $-36{\cdot}47$ kcal. for gaseous acetone and $-41{\cdot}57$ kcal. for gaseous *iso*propanol. Hence:

$$\textit{iso}\text{propanol}\,(g) = \text{acetone}\,(g) + \text{H}_2\,(g);$$
$$\Delta G^0 = 5{\cdot}10 \text{ kcal.} \quad (11)$$

This is close to the value of 5·035 kcal. which Cubberley & Mueller (1946) have obtained by extrapolation from the data of Kolb & Burwell (1945) for the equilibrium in the presence of a cupric oxide catalyst at 143–218°. It seems unlikely

that the mean of these ΔG^0 values is in error by more than 0·1 kcal. The mean value and partial vapour pressure data (Beare *et al.* 1930; Butler *et al.* 1935) give:

*iso*propanol (aq.) = acetone (aq.) + H_2 (*g*);
$\Delta G^0 = 5{\cdot}89$ kcal. (12)

From the ΔG^0 values for reactions (4) and (12) the value of $5{\cdot}22 \pm 0{\cdot}2$ kcal. is obtained for reaction (10). This agrees with that obtained from the ethanol-acetaldehyde reaction and corresponds to $E'_0 = -0{\cdot}320 \pm 0{\cdot}004$ V. at pH 7 and 25°. For the same conditions, Borsook (1940) obtained the value of −0·277 by calculation from potentiometric data for the alanine-ammonium pyruvate reaction (Wurmser & Filitti-Wurmser, 1938) and equilibrium data for the alanine-glutamic transaminase (Cohen, 1939) and the glutamic dehydrogenase reactions (v. Euler, Adler, Günther & Das, 1938). These data include at least two major uncertainties. First, the E'_0 value reported for the alanine-ammonium pyruvate reaction (−0·048 V. at pH 7 and 37°) is almost certainly too positive since, as a result of more extensive measurements, Filitti-Wurmser & Morel (1940) give −0·080 V. Secondly, whereas v. Euler *et al.* (1938) consider that under their experimental conditions α-iminoglutarate is the major product of the dehydrogenation of glutamate, recent measurements of Olsen & Anfinsen (1953) indicate that at equilibrium the product is almost wholly ammonium α-ketoglutarate.

ΔG^0 for the reduction of TPN. Olsen & Anfinsen (1953) have measured the equilibria with DPN and also with TPN in the glutamic dehydrogenase system. They found $K_c = 1{\cdot}45 \times 10^{-13}$ with DPN and $0{\cdot}99 \times 10^{-13}$ with TPN. Assuming that the thermodynamic equilibrium constants (K_a) for the two nucleotides are in the same ratio, ΔG^0 for the reduction of TPN may be obtained from the data for DPN, i.e. $\Delta G^0 = 5{\cdot}22 + RT \ln (1{\cdot}45/0{\cdot}99) = 5{\cdot}44$ kcal. Insufficient data are available to make an accurate estimate of the error involved in this assumption, but it probably does not amount to more than $\pm 0{\cdot}4$ kcal. The ΔG^0, $\Delta G'$ and E'_0 values for the dehydrogenation of reduced DPN and TPN, ethanol and *iso*propanol are given in Table 3.

Dehydrogenation of malate, glycerol 1-phosphate, lactate, glyceraldehyde phosphate and isocitrate. ΔG^0 values for these reactions (Table 3) have been calculated from ΔG^0 for the reduction of DPN or TPN and the equilibrium in the presence of the appropriate dehydrogenase. For the malate and glycerol phosphate systems the equilibrium constants are those obtained in this paper extrapolated to zero ionic strength. The equilibrium constant for the glyceraldehyde phosphate system is obtained from the measurements of Meyerhof & Oesper (1947). The value used ($K_c = 8 \times 10^{-9}$) was obtained at low ionic strengths (approx. 0·02; see Figs. 1 and 2 of Meyerhof & Oesper's paper). For the lactate-pyruvate system, Racker's (1950) value for the equilibrium at 25° has been used in preference to that of Kubowitz & Ott (1943) for 22°. If the latter value is corrected to 25° by the data given by Kubowitz & Ott for the effect of temperature on the equilibrium, the value of ΔG^0 is 0·2 kcal. greater than that obtained from Racker's data. The values for the *iso*citrate systems are obtained from Ochoa's (1948) measurements made at a low ionic strength (approx. 0·04).

Most of the ΔG^0 values in Table 3 may be taken to be correct to $\pm 0{\cdot}3$ kcal. The values for the reduction of TPN and the *iso*citrate reactions may be less accurate.

Oxidation-reduction potential measurements. ΔG^0 values for most of the above dehydrogenations have previously been obtained from potential measurements. As will be seen from Table 4, the values obtained from potential measurements are considerably more positive than those evaluated in this paper from equilibrium and thermochemical data.

In the potential measurements, it was necessary to add a reversibly reducible dye to act as a mediator between the enzyme system and the electrode. It is noteworthy that the largest discrepancies occur when indigodisulphonate was the mediator

Table 3. *Free-energy data for some biological dehydrogenations*

(Aqueous solution. Temp., 25°.)

Reductant	Oxidant	ΔG^0 kJ.	ΔG^0 kcal.	At pH 7: $\Delta G'$ (kcal.)	At pH 7: E'_0 (V.)
$DPNH + H^+$	DPN^+	−1·25	−5·22	4·33	−0·320
$TPNH + H^+$	TPN^+	−1·30	−5·44	4·11	−0·324
Ethanol	Acetaldehyde	2·31	9·68	9·68	−0·204
*iso*Propanol	Acetone	1·41	5·89	5·89	−0·296
L-Lactate$^-$	Pyruvate$^-$	2·47	10·32	10·32	−0·190
L-Malate^{2-}	Oxaloacetate^{2-}	2·74	11·45	11·45	−0·166
L-Glycerol 1-phosphate^{2-}	Dihydroxyacetone phosphate^{2-}	2·45	10·24	10·24	−0·192
Glyceraldehyde 3-phosphate^{2-} + HPO_4^{2-}	Glyceroylphosphate 3-phosphate^{4-}	1·39	5·83	5·83	−0·286
*dextro-iso*Citrate^{3-}	Oxalosuccinate^{3-}	0·89	3·74	3·74	−0·332
*dextro-iso*Citrate^{3-} + H^+	α-Ketoglutarate^{2-}	−3·03	−12·69	−3·14	−0·482

CORRIGENDUM

Biochemical Journal, Vol. 54, No. 1

The free-energy changes for the reduction of diphosphopyridine nucleotide and the dehydrogenation of L-malate and L-glycerol and 1-phosphate

By K. Burton and T. H. Wilson

Volume 54 (1953), No. 1

The free-energy changes associated with the individual steps of the tricarboxylic acid cycle, glycolysis and alcoholic fermentation and with the hydrolysis of the pyrophosphate groups of adenosinetriphosphate

By K. Burton and H. A. Krebs

Volume 54 (1953), No. 1

In the above papers an error has been made in the conversion of calories into joules. In accordance with the proposal of the Royal Society some of the more important values in the tables were given both in kilocalories and kilojoules (in the text kilocalories were used throughout) and in the calculations the calorie values have been divided, instead of multiplied by, 4·1840.

The following alterations are required:

p. 93, col. 2, line 5 of paragraph 2 of Summary: *for* 1·25 kJ. *read* 21·84 kJ.

p. 95, col. 2, line 1: *for* 0·3261 kJ. *read* 5·708 kJ.

p. 96, col. 4, Table 1, fourth line from end: *for* 2·90 kJ. *read* 50·79 kJ.

Same table, same col. last line: *for* 13·16 kJ. *read* 237·19 kJ.

Also p. 99, Table 1, head of columns: *for* $\Delta G°f$ *read* $-\Delta G°f$.

The following table columns are to be replaced by the figures given below. These are so printed that they can be stuck over the original.

p. 92, Table 3, first column of figures	p. 96, Table 1, last column of figures	p. 99, Table 4, last colomn of figures	p. 101, Table 5, last column of figures	p. 105, Table 7, last column of figures
−21·84	141·04	481·16	−276	−134·72
−22·76	399·49	900·27	+7·5	−116·73
40·50	372·33	1145·10	+23·14	−233·89
24·64	161·17	1139·34	+8·54	+16·32
43·18	373·38	506·26	−1·88	+7·20
47·91	371·08	798·06	−215·43	−2·30
42·84	371·66*	517·31	−68·74	+2·09
24·39	371·33†	845·08	−35·56	+21·76
15·65	26·65	797·18	−286·56	+23·05
−53·09	79·50	1123·70	−151·17	+7·66
	721·45	474·13	−3·68	+46·23
	699·19		−189·28	−19·87
	171·84		−177·8	+4·44
	381·58		+15·5	−2·68
	353·97		−2·9	−60·67
	386·02		−1183·4	−65·02
	586·97		−259·03	−61·30
	181·54		−2871·56	−62·34
	647·14		−182·55	+3·77
	604·21		−177·49	
	917·89		−182·30	
	719·65		+76·48	
	695·00		−0·75	
	488·52		−26·74	
	—			
	746·38			
	690·23			
	—			

Table 4. *Comparison between ΔG^0 values evaluated in this paper and those obtained from potential measurements*

Reaction (aqueous solution)	Evaluated in this paper ΔG^0 (25°) (kcal.)	Evaluated from potential measurements ΔG^0 (kcal.)	Reference
Ethanol-acetaldehyde	9·68	16·42 (30°)	Lehmann (1934)
*iso*Propanol-acetone	5·89	8·13 (35°)	Wurmser & Filitti (1936)
Lactate-pyruvate	10·32	14·58 (32°)	Baumberger *et al.* (1933)
		11·6 (37°)	Wurmser & Mayer-Reich (1933)
		11·35 (37°)	Banga, Laki & Szent-Györgyi (1933)
		11·44 (35°)	Barron & Hastings (1934)
		11·76 (25°)	
Malate-oxaloacetate	11·45	12·1 (37°)	Laki (1937)
		15·2 (38°)	Lehmann & Hoff-Jörgensen (1939)
		15·65 (25°)	
		12 to 15·4 (25°)	Burton*

* These unpublished measurements were made using purified malic dehydrogenase and diaphorase in the presence of nile blue or phenosafranine as mediator. The results, though not reproducible, were all in the range indicated.

(Lehmann, 1934; Baumberger, Jürgensen & Bardwell, 1933; Lehmann & Hoff-Jörgensen, 1939). The measurements of Baumberger *et al.* (1933) have been criticized by Wurmser & Mayer-Reich (1933) and by Barron & Hastings (1934) on the basis that the potential of the indigo disulphonate ($-0{\cdot}125$ V. at pH 7 and 30°) is too positive.

The discrepancies between the potentiometric values and those obtained in this paper would be explained if thermodynamic equilibrium had not been attained in the potential measurements. Of the reactions studied in this way, there is only the succinate-fumarate reaction for which the evidence is really convincing that equilibrium was attained (Borsook & Schott, 1931).

SUMMARY

1. The reaction between *iso*propanol and diphosphopyridine nucleotide (DPN) to form acetone and reduced DPN is catalysed by crystalline preparations of yeast alcohol dehydrogenase. About 500 times as much enzyme are required to obtain the same rate of reaction as that with the ethanol-DPN-acetaldehyde system.

2. The equilibrium constant of the isopropanol-DPN-acetone reaction has been combined with reliable free-energy data for *iso*propanol and acetone to calculate the free-energy change for the reduction of DPN. $\Delta G^0 = 5{\cdot}22$ kcal. or 1·25 kJ. at 25°. This value corresponds to an oxidation-reduction potential E'_0 of $-0{\cdot}320$ V. at pH 7.

3. The equilibria of the L-malic and L-glycerol 1-phosphate dehydrogenase systems have been measured at low ionic strengths.

4. These and other equilibrium data have been combined with the data for DPN to obtain reliable values (summarized in Table 3) for the free-energy changes for the reduction of triphosphopyridine nucleotide and the dehydrogenations of L-malate, L-lactate, L-glycerol 1-phosphate, glyceraldehyde 3-phosphate and *iso*citrate.

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REFERENCES

Banga, I., Laki, K. & Szent-Györgyi, A. (1933). *Hoppe-Seyl. Z.* **217**, 43.
Baranowski, T. (1949). *J. biol. Chem.* **180**, 535.
Barron, E. S. G. & Hastings, A. B. (1934). *J. biol. Chem.* **107**, 567.
Baumberger, J. P., Jürgensen, J. J. & Bardwell, K. (1933). *J. gen. Physiol.* **16**, 961.
Beare, W. G., McVicar, G. A. & Ferguson, J. B. (1930). *J. phys. Chem.* **34**, 1310.
Borsook, H. (1940). *J. biol. Chem.* **133**, 629.
Borsook, H. & Schott, H. F. (1931). *J. biol. Chem.* **92**, 535
Burton, K. (1952). *Biochim. biophys. Acta*, **8**, 114.
Burton, K. & Krebs, H. A. (1953). *Biochem. J.* **54**, 94.
Butler, J. A. V., Ramchandani, C. N. & Thomson, D. W. (1935). *J. chem. Soc.* p. 280.
Clark, W. M. (1938). *J. appl. Phys.* **9**, 97.
Cohen, P. P. (1939). *Biochem. J.* **33**, 1478.
Cubberley, A. H. & Mueller, M. B. (1946). *J. Amer. chem. Soc.* **68**, 1149.
Dirscherl, W. & Nahm, H. (1940). *Ber. dtsch. chem. Ges.* **73** B, 448.
Dixon, M. (1949). *Multi-Enzyme Systems*, 1st ed. Cambridge University Press.
Euler, H. von, Adler, E. & Günther, G. (1937). *Hoppe-Seyl. Z.* **249**, 1.

Euler, H. von, Adler, E., Günther, G. & Das, N. B. (1938). *Hoppe-Seyl. Z.* **254**, 61.
Filitti-Wurmser, S. & Morel, M. (1940). *Enzymologia*, **9**, 369.
Gibson, K. S. & Balcom, M. M. (1947). *J. Res. nat. Bur. Stand.* **38**, 601.
Gilson, L. E. (1932). *J. Amer. chem. Soc.* **54**, 1445.
Green, D. E. & Dewan, J. G. (1937). *Biochem. J.* **31**, 1069.
Horecker, B. L. & Kornberg, A. (1948). *J. biol. Chem.* **175**, 385.
Hoskins, W. M., Randall, M. & Schmidt, C. L. A. (1930). *J. biol. Chem.* **88**, 215.
Johnson, M. J. (1949). In Lardy, H. A., *Respiratory Enzymes*, 2nd ed. Minneapolis: Burgess Publishing Co.
Kelley, K. K. (1929). *J. Amer. chem. Soc.* **51**, 1145.
Kolb, H. J. & Burwell, R. L. (1945). *J. Amer. chem. Soc.* **67**, 1084.
Krebs, H. A. & Eggleston, L. V. (1945). *Biochem. J.* **39**, 408.
Kubowitz, F. & Ott, P. (1943). *Biochem. Z.* **314**, 94.
Laki, K. (1937). *Hoppe-Seyl. Z.* **249**, 63.
Lehmann, J. (1934). *Biochem. Z.* **274**, 321.
Lehmann, J. & Hoff-Jörgensen, E. (1939). *Skand. Arch. Physiol.* **82**, 113.
LePage, G. A. (1949). In Carter, H. E., *Biochemical Preparations*. New York: John Wiley and Sons Inc.
Lewis, G. N. & Randall, M. (1923). *Thermodynamics*, 1st ed. New York: McGraw-Hill Book Co. Inc.
McDonald, M. R. (1949). *J. gen. Physiol.* **32**, 33.
Mehler, A. H., Kornberg, A., Grisolia, S. & Ochoa, S. (1948). *J. biol. Chem.* **174**, 961.
Meyerhof, O. & Junowicz-Kocholaty, R. (1943). *J. biol. Chem.* **149**, 71.
Meyerhof, O. & Oesper, P. (1947). *J. biol. Chem.* **170**, 1.
Miles, C. B. & Hunt, H. (1941). *J. phys. Chem.* **45**, 1346.
Müller, D. (1934). *Biochem. Z.* **268**, 152.
Negelein, E. & Wulff, H. J. (1937). *Biochem. Z.* **293**, 351.
Ochoa, S. (1948). *J. biol. Chem.* **174**, 133.
Olson, J. A. & Anfinsen, C. B. (1953). *J. biol. Chem.* (in the Press).
Parks, G. S., Moseley, J. R. & Peterson, P. V. (1950). *J. chem. Phys.* **18**, 152.
Racker, E. (1947). *J. biol. Chem.* **167**, 843.
Racker, E. (1950). *J. biol. Chem.* **184**, 313.
Roberts, J. D. & Green, C. (1946). *J. Amer. chem. Soc.* **68**, 214.
Schlenk, F., Hellström, H. & Euler, H. von (1938). *Ber. dtsch. chem. Ges.* **71**, 1471.
Stern, J. R., Ochoa, S. & Lynen, F. (1952). *J. biol. Chem.* **198**, 313.
Straub, F. B. (1939). *Biochem. J.* **33**, 787.
Straub, F. B. (1942). *Hoppe-Seyl. Z.* **275**, 63.
Theorell, H. & Bonnischen, R. K. (1951). *Acta. chem. scand.* **5**, 1105.
Wurmser, R. & Filitti, S. (1936). *C.R. Acad. Sci., Paris*, **212**, 1848.
Wurmser, R. & Filitti-Wurmser, S. (1938). *C.R. Soc. Biol., Paris*, **128**, 133.
Wurmser, R. & Mayer-Reich, N. (1933). *C.R. Acad. Sci., Paris*, **196**, 612.

The Free-energy Changes Associated with the Individual Steps of the Tricarboxylic Acid Cycle, Glycolysis and Alcoholic Fermentation and with the Hydrolysis of the Pyrophosphate Groups of Adenosinetriphosphate

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Now that the main stages of the energy-producing reactions in living matter are known, investigations into biological energy transformations are likely to become increasingly concerned with the mechanisms by which the free energy of respiration and fermentation is converted into work. In many of such studies it is essential to possess accurate data on the free-energy changes associated with the individual steps of the energy-giving reactions and the hydrolysis of energy-rich phosphate compounds. Reliable data for many of these reactions are still lacking. Many free-energy data which have hitherto been used, e.g. by Lipmann (1941, 1946), Kalckar (1941), Ogston & Smithies (1948), Dickens (1951), Ochoa (1951), Kaplan (1951), Ogston (1951) and Oesper (1951) are not more than approximate assessments.

The preceding papers report new equilibrium measurements for the fumarase, aconitase and transaminase enzyme systems (Krebs, 1953*a*, *b*) and certain nicotinamide nucleotide dehydrogenase systems (Burton & Wilson, 1953). In this paper, the free-energy changes calculated from these and other equilibrium measurements are used in conjunction with free-energy data derived from literature data on heats of combustion and heat capacities. The newer data make it possible to evaluate more accurately than before the free-energy changes associated with each step of the biological degradation of carbohydrate.

Section 1 is concerned with the critical selection of thermochemical and ancillary data for pure substances of biological interest in order to calculate the