- 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-Jørgensen, 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. dtsh. 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

best available values for the free energies of formation. In section 2 the free-energy changes are evaluated for the steps of the tricarboxylic acid cycle, in section 3 for the hydrolysis of the terminal phosphate groups of adenosinetriphosphate (ATP) and in section 4 for the glycolytic reactions.

THERMODYNAMIC SYMBOLS AND CONVENTIONS

The symbols used in this paper are based on the conventions of Lewis & Randall (1923) and the Report of the Royal Society Committee on Symbols (1951). They are as follows:

ΔG	Increment of free energy for the given reaction under specified conditions (ΔF of Lewis & Randall, 1923).
ΔG^0	ΔG for standard conditions, i.e. the reactants in their standard states.
$\Delta G'$	ΔG for a reaction in solution with all reactants except the hydrogen ion in their standard states.
ΔG^0_f	Free energy of formation of a compound from its elements under standard conditions.
ΔH^0_f	Heat of formation of a compound under standard conditions.
ΔS^0_f	Entropy of formation of a compound under standard conditions.
K_a	Thermodynamic equilibrium constant, i.e. the product of the activities of the reactants on the right-hand side divided by the corresponding product for the left-hand side when the reaction is at equilibrium.
K_c	Equilibrium constant based on concentrations instead of activities.
I	Ionic strength.
m	Molality (moles/1000 g. water).
γ	Molal activity coefficient (i.e. activity/molality).
R	Gas constant (8.3144 abs. joules/deg./mole or 1.9872 cal./deg./mole).
T	Absolute temperature.

Some of the more important values in the tables are given both in kilocalories (kcal.) and in kilojoules (kJ.), but in the text the kilocalorie is used throughout. By definition 1 thermodynamical calorie is equal to 4.1840 absolute joules. The standard states (unit activity) are a defined physical form for pure substances (e.g. liquid water) and the ideal molal solution for dissolved reactants. The relation between ΔG^0 and K_a is

$$\Delta G^0 = -RT \ln K_a.$$

At 25°, $\Delta G^0 = -(1.3643 \log K_a)$ kcal. or $-(0.3261 \log K_a)$ kJ. $\Delta G = \Delta G^0 + RT \ln$ product of activities of reactants on right-hand side/product of activities of reactants on left-hand side. Unless otherwise stated, the data refer to 25°. All free-energy changes are expressed per mole. In accordance with the usual custom, most of the free-energy values in this paper are given to 0.01 kcal. Although some of the equilibrium data justify this order of accuracy, most of the values which are based on thermal measurements are not certain to the first decimal place (0.1 kcal.). However, two decimal places are retained for the purposes of calculation.

SECTION 1

Thermochemical free-energy data

The standard free energies of formation for several substances of physiological importance are given in Table 1. These have been obtained from what appear to be the most reliable thermochemical and ancillary data which are available. In most cases the free energy of formation (ΔG^0_f) for the pure compound is obtained by the relation

$$\Delta G^0_f = \Delta H^0_f - T\Delta S^0_f,$$

where ΔH^0_f and ΔS^0_f respectively are the heats and entropies of formation, and T is the absolute temperature. ΔH^0_f and ΔS^0_f have been calculated from data for the heat of combustion and the entropy using values for ΔH^0_f of CO_2 and H_2O and the entropies of the elements in the standard states as given by Rossini, Wagman, Evans, Levine & Jaffe (1952). Where no value is given for ΔS^0_f , ΔG^0_f has been evaluated from ΔH^0_f and spectroscopic data. The value for ΔH^0_f of carbon dioxide differs from that used by earlier authors (e.g. Parks & Huffman, 1932; Borsook & Huffman, 1938) by 0.19 kcal.; this accounts for some of the differences between the ΔG^0_f values obtained here and those previously given for the same compounds.

Reliable thermochemical data are also available for other amino-acids and peptides (Borsook & Huffman, 1938; Huffman, 1942), several purines (Stiehler & Huffman, 1935) and a number of sugars (Clarke & Stegeman, 1939; Anderson & Stegeman, 1941; Jack & Stegeman, 1941).

Sources of the data. Those for acetaldehyde, acetone and ethanol are as previously described (Burton, 1952).

There is considerable uncertainty (± 1 kcal.) in the available data for the heat of combustion of acetic acid; the value used is that selected by Bichowsky & Rossini (1936). The entropy value of acetic acid is that of Parks & Huffman (1932).

ΔG^0 for the solution of acetic acid in water was calculated from the vapour pressure measurements of Fredenhagen & Liebster (1932) and data given by Halford (1941) for the equilibrium between dimeric and monomeric acetic acid in the vapour phase.

Table 1. *Thermochemical free-energy data*

(All values are per mole of substances at 25°. Unless otherwise stated, data for pure compounds refer to the crystalline material.)

Compound	$-\Delta H^{\circ f}$ (kcal.)	$-\Delta S^{\circ f}$ (cal./deg.)	$-\Delta G^{\circ f}$ (kcal.)	ΔG° of solution in water (kcal.)	ΔG° of ionization (kcal.)	$-\Delta G^{\circ f}$ in aqueous solution	
						(kcal.)	(kJ.)
Acetaldehyde (gas)	39.76	—	31.96	-1.75	—	33.71	8.06
Acetic acid (liq.)	116.44	76.1	93.75	-1.73	—	95.48	22.82
Acetate	—	—	—	—	6.49	88.99	21.27
Acetone (liq.)	59.34	74.32	37.18	-1.34	—	38.52	9.21
D,L-Alanine	134.72	153.7	88.89	-0.35	—	89.24	21.33
L-Alanine	134.17	153.7	88.34	-0.35	—	88.69	21.20
						88.83*	21.23*
						88.75†	21.21†
NH ₃ (gas)	—	—	3.98	-2.39	—	6.37	1.49
NH ₄ ⁺	—	—	—	—	-12.63	19.00	4.54
L-Aspartic acid	233.75	194.1	174.88	2.45	—	172.43	41.21
L-Aspartate ²⁻	—	—	—	—	5.32	167.11	39.94
n-Butanol	79.59	131.5	40.39	-0.68	—	41.07	9.81
Butyric acid (liq.)	128.09	125.2	90.65	-0.55	—	91.2	21.8
Butyrate	—	—	—	—	6.58	84.6	20.2
CO ₂ (gas)	94.052	—	94.26	2.00	—	92.26	22.05
HCO ₃ ⁻	—	—	—	—	8.66	140.29	33.53
Ethanol (liq.)	66.34	82.4	41.77	-1.62	—	43.39	10.37
Fumaric acid	194.13	126.2	156.49	1.82	—	154.67	36.97
Fumarate ²⁻	—	—	—	—	10.26	144.41	34.51
α-D-Glucose	304.64	291.7	217.56	-1.82	—	219.38	52.43
L-Glutamic acid	240.38	222.4	174.05	2.05	—	172.00	41.11
L-Glutamate ²⁻	—	—	—	—	5.89	166.11	39.70
Glycerol (liq.)	159.80	153.6	114.02	-2.74	—	116.76	27.91
Methane (gas)	17.89	—	12.14 (2.90 kJ.)	—	—	—	—
Succinic acid	224.92	155.1	178.68	0.29	—	178.39	42.64
Succinate ²⁻	—	—	—	—	13.42	164.97	39.43
Water (liq.)	68.317	—	56.69 (13.16 kJ.)	—	—	—	—

* Calculated from value for DL-alanine.

† Value adopted in this paper.

The combustion and entropy data for alanine, aspartic acid and glutamic acid and also ΔG^0 for the solution of L- and DL-alanine are those given by Borsook & Huffman (1938). It is noteworthy that the ΔG^0 of aqueous L-alanine is within 0.14 kcal. of that obtained from the ΔG^0 of aqueous DL-alanine and ΔG^0 for the racemization ($-RT \ln 2 = 0.41$ kcal.). The mean (-88.75 kcal.) of the two values for aqueous L-alanine is adopted for subsequent calculations.

The ΔG^0 values for the solution and ionization of aspartic and glutamic acid differ from those given by Borsook & Huffman (1938); they are obtained as follows: at 25° a saturated solution of L-aspartic acid contains 0.0375 mole/1000 g. water (see Borsook & Huffman, 1938) and from the relevant ionization constant (1.24×10^{-4}), 95 % or 0.0355 molal remains undissociated. Hoskins, Randall & Schmidt (1930) have measured the activity coefficient (γ) of aspartic acid in aqueous solution at 0° and at concentrations up to $m = 0.03$. The curve obtained by plotting $\log \gamma$ against m has been extrapolated to $m = 0.0355$. Hence γ for the undissociated acid is found to be 0.45. By applying the relation $d \ln \gamma/dT = -(\bar{H} - \bar{H}^0)/RT^2$, where $(\bar{H} - \bar{H}^0)$ is the relative

partial molal heat content (190 cal./mole; Borsook & Huffman, 1938) it will be seen that the temperature correction to 25° is negligible. ΔG^0 of solution is therefore $-RT \ln \gamma m = -RT \ln (0.45 \times 0.0355) = 2.45$ kcal. The value obtained by Borsook & Huffman is 2.55 kcal. To obtain ΔG^0 of ionization, the thermodynamic ionization constant has been taken to be 1.24×10^{-4} corresponding to $\text{pK} = 3.90$ (Schmidt, 1938; Batchelder & Schmidt, 1940; Smith & Smith, 1942).

The procedure for L-glutamic acid is similar. The saturated solution has been taken to be 0.0595 molal at 25° and the acid to be 96.2% undissociated. The activity data of Hoskins *et al.* (1930) have been extrapolated to 0.057*m* undissociated acid, where γ is found to be 0.55. Hence ΔG^0 of solution is $-RT \ln (0.55 \times 0.057) = 2.05$ kcal. The value obtained by Borsook & Huffman is 2.3 kcal. To calculate ΔG^0 of ionization the appropriate pK has been taken as 4.315 (Neuberger, 1936).

The data for NH_3 and NH_4^+ are from Rossini *et al.* (1952). For *n*-butanol the heat of combustion is Rossini's (1934) value and the entropy value used is that given by Parks &

Huffman (1932). ΔG^0 of solution is obtained from data of Butler, Ramchandani & Thomson (1935). The combustion and entropy data for butyric acid are those selected by Parks & Huffman (1932); ΔG^0 of solution is an estimated value and ΔG^0 of ionization is obtained from data of Saxton & Darken (1940).

The ΔG^0f and ΔH^0f values for carbon dioxide, water and methane are from Rossini *et al.* (1952). ΔG^0 for the solution of CO_2 is obtained from the solubility data of Harned & Davis (1943). ΔG^0f for the HCO_3^- ion is calculated from the values for water and aqueous carbon dioxide and the ionization constant as measured by Harned & Davis.

The combustion and entropy data used for succinic and fumaric acids are those given by Huffman & Fox (1938). The ΔG^0 values for solution and ionization are those of Borsook & Schott (1931); in the case of succinic acid more recent measurements of the ionization constants (Pinching & Bates, 1950) and of the activity coefficients in aqueous solution (Robinson, Smith & Smith, 1942) confirm the data used by Borsook & Schott.

For glucose the combustion and entropy data for anhydrous α -D-glucose given by Huffman & Fox (1938) are used. When glucose crystallizes from aqueous solution at 25°, the solid form obtained is the monohydrate and not the anhydrous form. Huffman & Fox (1938) extrapolated the solubility data for the anhydrous form at 50–100° (Jackson & Silsbee, 1922) and obtained the value of 0.162 for the mole ratio of glucose (moles glucose/mole water) in an aqueous solution which is in equilibrium with the anhydrous form at 25°. In order to obtain ΔG^0 of solution it is necessary to know the activity coefficient (γ) of glucose in this solution. An approximate value for γ may be obtained by extrapolating data for lower concentrations which may be calculated (Table 2) from the osmotic pressure measurements of Berkeley & Hartley (1906). A straight line is obtained by plotting $1/\gamma$ against the mole ratio. By extrapolation, $\gamma=2.4$ at a mole ratio of 0.162. Hence for the solution of anhydrous glucose at 25°,

$$\Delta G^0 = -RT \ln (2.4 \times 0.162/0.0180) = -1.82 \text{ kcal.}$$

(0.0180 is the mole ratio of a molal solution).

Table 2. *Activity of glucose in aqueous solution at 0°*

(Calculated from osmotic pressure data of Berkeley & Hartley (1906). Concentrations per 100 ml. have been converted to mole ratios with the aid of density data of Jackson (1917). For water, unit activity is the pure liquid; for glucose, it is the ideal molal solution. The activity of the glucose was calculated from that of the water by the method of Lewis & Randall (1923).)

Glucose concn. (g./100 ml.)	Osmotic pressure (atm.)	Mole ratio of glucose	Activity of water	Activity coefficient of glucose
9.98	13.21	0.0106	0.989	1.02
19.95	29.17	0.0226	0.976	1.11
31.92	53.19	0.0401	0.958	1.16
44.82	87.87	0.063	0.930	1.30
54.86	121.18	0.085	0.907	1.38

The data for glycerol are derived from the heat of combustion of Parks, West, Naylor, Fujii & McClaine (1946) and the entropy value given by Ahlberg, Blanchard & Lundberg (1937). ΔG^0 of solution is obtained from vapour pressure data for aqueous solutions of glycerol. Since the vapour

pressure of 98.43 % glycerol is 0.536 mm. Hg at 13° (Daudt, 1923) while that of anhydrous glycerol is 0.0025 mm. Hg at 50° (Stedman, 1928), less than 0.5 % of the vapour pressure of the 98.43 % solution is due to the glycerol and the remainder is due to the water component. Hence, if p is the vapour pressure of the solution, and p_0 that of pure water at the same temperature, the relative vapour pressure (p/p_0) may be taken as the thermodynamic activity of the water present in the glycerol solution. This has been evaluated for a range of glycerol concentrations using vapour pressure data of Scatchard, Hamer & Wood (1938) for solutions up to 56.5 % glycerol, those of Fricke (1929) for higher concentrations (up to 98 %) and the value of Daudt for 98.43 % glycerol. When p/p_0 is plotted against the glycerol concentration, these measurements are very consistent, not only with each other, but also with those of Grover & Nicol (1940) at 25° and the values of Dieterici (at 0°) as compiled in the *International Critical Tables* (1931). They do not agree with the values of Drucker & Moles (1909) nor with the values given by Dulitskaya (1945) for 25°. In contrast to the other data neither of these two series of results falls on a smooth curve. The activity of glycerol in aqueous solution is obtained from the activity of the solvent by integration according to the procedure of Lewis & Randall (1923). At 15°, the value obtained for the activity of anhydrous glycerol is 111 times that of an ideal molal solution in water. ΔG^0 of solution is therefore $-RT \ln 111 = -2.695$ kcal. The value at 25° (2.74 kcal.) is obtained from this and the heat of solution (-1.38 kcal./mole; Fricke, 1929). Any uncertainty in this value is mainly due to errors in the data for the higher concentrations (>90 %) of glycerol. Even if the error at these concentrations amounted to 50 % of the vapour pressure the error in ΔG^0 would be only 0.07 kcal. The values used for the activity of both water and glycerol in solutions more concentrated than 0.25 mole glycerol/mole water are given in Table 3. Data for lower concentrations are given by Scatchard *et al.* (1938).

Table 3. *Activity of glycerol in aqueous solution*

(Based mainly on vapour pressure data of Fricke (1929) for 15°. p/p_0 = vapour pressure of solution relative to that of pure water. For water, unit activity is pure water; for glycerol, it is the ideal molal solution.)

Mole ratio of glycerol	p/p_0 = Activity of water	Activity of glycerol
0.250	0.763	14.9
0.30	0.724	18.4
0.40	0.650	25.0
0.50	0.595	30.6
0.70	0.498	41.1
1.00	0.422	50.3
1.50	0.312	64
2.00	0.264	71
5.0	0.124	91
10.0	0.062	101
∞	—	111

Accuracy of the ΔG^0f values. It is difficult to give precise limits of the errors involved, but it is useful to consider the following estimates when the data are used in subsequent calculations.

The reliability of the ΔG^0f values depends primarily on the accuracy with which the heat of

combustion has been measured. In the case of glucose an error of 0.03 % in the heat of combustion would introduce an error of 0.2 kcal. into ΔH^0_f and hence into ΔG^0_f . An error of 0.5 % in the entropy would also introduce an error of 0.07 kcal. into ΔG^0_f . Except for the heat of combustion of acetic acid and possibly that of butyric acid, the combustion and entropy data are of at least this order of accuracy. The simpler compounds (ammonia, carbon dioxide, water and methane) have been very carefully studied, and in each case the error is almost certainly less than 0.05 kcal. The uncertainties in the ΔG^0_f values for aqueous glucose, aspartic and glutamic acids and the aspartate and glutamate ions are probably about ± 0.4 kcal. The values given for the other compounds, with the exception of acetic and butyric acids, are likely to be correct to ± 0.2 kcal.

Inconsistencies in the free-energy data

Some apparent inconsistencies have come to light during this work and require further investigation. They are as follows:

Formate. ΔG^0_f as evaluated from Woods's (1936) measurements of the formic hydrogenlyase equilibrium is -83.77 kcal. whereas -80.0 kcal. is obtained from thermochemical and ancillary data (Rossini *et al.* 1952).

Sucrose. Data for the heat of combustion (Clarke & Stegeman, 1939) and entropy (Parks, Huffman & Barmore, 1933), together with those for ΔG^0 of solution (Scatchard *et al.* 1938), give the value of -370.90 kcal. for ΔG^0_f of aqueous sucrose.

This value combined with that for aqueous glucose and ΔG^0 for the conversion of glucose to fructose (0.44 kcal. see section 4) gives $\Delta G^0 = -10.8$ kcal. for the inversion of sucrose. However, Doudoroff's (1943) equilibrium data for the sucrose phosphorylase reaction, together with the value of ΔG^0 obtained for the hydrolysis of glucose 1-phosphate (see section 4), indicate that ΔG^0 of inversion is -6.5 kcal. It is unlikely that the discrepancy can be accounted for by errors in the combustion measurements. The values used for the heats of combustion of sucrose and of glucose, together with the value of Clarke & Stegeman (1939) for fructose and data for the heats of solution of anhydrous glucose (Hendricks, Dorsey, LeRoy & Moseley, 1930), fructose (Hendricks, Steinbach, LeRoy & Moseley, 1934) and sucrose (Higbie & Stegeman, 1950), indicate that ΔH of inversion is -4.9 kcal.; this is in fair agreement with the measured value of -3.56 kcal. (Sturtevant, 1937).

Lactose. Two independent but inconsistent values for ΔG^0_f of aqueous lactose may be obtained starting from the combustion and entropy data for α -lactose monohydrate and anhydrous β -lactose. The data of Clarke & Stegeman (1939) and of Anderson & Stegeman (1941) give -419.11 kcal. for ΔG^0_f of the solid α -lactose monohydrate and -374.56 kcal. for solid anhydrous β -lactose. The solubility data for the two forms (Hudson, 1908) indicate that ΔG^0 of solution to form the α - β equilibrium mixture is 0.27 kcal. for the monohydrate and about -0.7 kcal. for the anhydrous form. The data for the monohydrate give $(-419.11 + 0.27 - \Delta G^0_f \text{ of liquid water}) = -362.15$ kcal. for the ΔG^0_f value of aqueous

lactose, while the data for the anhydrous form give $-374.56 - 0.7 = -375.26$ kcal.

Maltose. The thermochemical measurements of Anderson & Stegeman (1941) and Clarke & Stegeman (1939) for β -maltose monohydrate, together with the solubility data of Dehn (1917), give the ΔG^0_f value of -357.80 kcal. for aqueous maltose. This indicates that ΔG^0 for the hydrolysis of aqueous maltose is -23.47 kcal. However, the equilibrium data of Monod & Torriani (1950) for the amylo-maltose system suggest that ΔG^0 for the hydrolysis of maltose is approximately the same as for the hydrolysis of a glucosidic link of starch. In section 4 of this paper this is estimated to be -4.2 kcal.

SECTION 2

Free energies of formation for intermediates of the tricarboxylic acid cycle and associated metabolites

As stated in section 1, ΔG^0_f values based on thermochemical measurements are available for two intermediates of the cycle, namely, succinate and fumarate, and also for three metabolites related to the cycle, namely, alanine, aspartate and glutamate (see Table 1). ΔG^0_f values for other intermediates of the cycle, obtained from the values for the above five substances, and equilibrium data are given in Table 4. Details of the procedure are as follows.

Activity coefficients. It is necessary in the calculations to consider the activity coefficients of the reactants in the equilibrium mixtures. For most non-electrolytes the values of these coefficients may be taken as very close to unity even at concentrations as high as 1M. If, as in many cases, the reactions involve electrolytes and the measurements were made at an ionic strength $I > 0.1$, the coefficients are expected to be markedly different from unity. In many of the reactions considered, ions of similar size, valency and charge distribution appear on opposite sides, e.g. the fumarase reaction

The activity coefficients may be assumed to be identical for similar ions in the same solution and the coefficients may in these cases be taken to cancel each other in the expression for the thermochemical equilibrium constant (K_a).

If the ionic strength is less than 0.05M, fairly reliable values can be assessed for the activity coefficients of most electrolytes, since at low ionic strengths the activity coefficients are almost identical for salts of the same valency type. If measurements of enzyme-catalysed equilibria are therefore carried out at low ionic strengths, the uncertainties due to activity coefficients are reduced to a minimum. However, some equilibrium measurements have been carried out in the presence of a buffer which may contribute as much as 0.1 to the ionic strength of the equilibrium mixture.

Fumarate. The accuracy of most of the free-energy data calculated in this section depends on the accuracy of the $\Delta G^{\circ}f$ value for fumarate which serves as a reference substance. The value given in Table 1 is obtained from modern thermochemical data, and its reliability is confirmed by the fact that it is reasonably consistent with free-energy data for both the succinic dehydrogenase and the aspartase reactions. For example, the potential measurements of Borsook & Schott (1931) for the reaction

indicate that ΔG° is 20.16 kcal., whereas the $\Delta G^{\circ}f$ values for succinate and fumarate (Table 1) give 20.56 kcal. The discrepancy of 0.40 kcal. is within the limits of error of the thermochemical and ancillary data but is somewhat larger

Table 4. Values for the free energy of formation ($\Delta G^{\circ}f$) for metabolites associated with the tri-carboxylic acid cycle

(The data refer to aqueous solution and 25° and are derived from equilibrium measurements and $\Delta G^{\circ}f$ values for other metabolites given in Table 1. The values for acetoacetate and β -hydroxybutyrate are less reliable than the other values.)

	$\Delta G^{\circ}f$	
	kcal.	kJ.
Acetoacetate ⁻	115	27.5
cis-Aconitate ³⁻	215.17	51.43
Citrate ³⁻	273.90	65.46
isoCitrate ³⁻	272.31	65.08
β -Hydroxybutyrate ⁻	121	28.9
α -Ketoglutarate ²⁻	190.74	45.59
Lactate ⁻	123.64	29.55
Malate ²⁻	201.98	48.27
Oxaloacetate ²⁻	190.53	45.54
Oxalosuccinate ²⁻	268.57	64.19
Pyruvate ⁻	113.32	27.08

than the uncertainty of the potentiometric ΔG° value (possibly 0.07 kcal.). Since it is uncertain what fraction of the error lies in the value for succinate and in that for fumarate, no correction is attempted. For the aspartase reaction

the $\Delta G^{\circ}f$ values of the reactants give $\Delta G^{\circ} = 3.70$ kcal. (25°), whereas Borsook & Huffman (1938) obtained 3.72 kcal. (37°) using the equilibrium data of Woolf (1929).

Malate, oxaloacetate, pyruvate and α -ketoglutarate. Starting from the value for fumarate $\Delta G^{\circ}f$ values may be obtained for malate and oxaloacetate. For the fumarase reaction (1) Krebs (1953a) found $K_e = 4.42$ at 25°. Assuming that the activity coefficients of fumarate and malate cancel out, $\Delta G^{\circ} = -RT \ln K_e = -0.88$ kcal. Hence, for malate²⁻, $\Delta G^{\circ}f = -144.41 - 56.69 - 0.88 = -201.98$ kcal. The value for oxaloacetate is obtained from the reaction

$\Delta G^{\circ} = 11.45$ kcal. (Burton & Wilson, 1953).

Data for the pyruvate⁻ and α -ketoglutarate²⁻ ions may be obtained (cf. Darling, 1945, 1947) with the help of equilibrium data for the transaminase systems

According to Krebs (1953b), at 25°, $K_e = 6.74$ for reaction (2) and 1.52 for reaction (3). These values are converted to thermodynamic equilibrium constants as follows. The measurements for reaction (2) were carried out at an ionic strength (I) of 0.152. The activity coefficients (γ) of the aspartate²⁻ and glutamate²⁻ ions in the equilibrium mixture are assumed to be the same as in solutions of the respective monosodium salts at the same ionic strength. These have been measured by Hoskins *et al.* (1930) for concentrations up to $m = 0.06$. The curve obtained by plotting $\log \gamma$ against $\sqrt{m}$ has been extrapolated to $m = I = 0.152$. The values of γ thus obtained are 0.655 for sodium aspartate and 0.615 for sodium glutamate respectively. Hence

$$\frac{\gamma_{\text{aspartate}^{2-}}}{\gamma_{\text{glutamate}^{2-}}} = \frac{(\gamma_{\text{sodium aspartate}})^2}{(\gamma_{\text{sodium glutamate}})^2} = 1.15.$$

For the α -ketoglutarate ion, γ is assumed to be the same as for the oxaloacetate ion. Hence,

$$\Delta G^{\circ} = -RT \ln (1.15 \times K_e) = -1.21 \text{ kcal.}$$

$\Delta G^{\circ}f$ for α -ketoglutarate is obtained from this value and the $\Delta G^{\circ}f$ values for glutamate, aspartate and oxaloacetate.

The equilibrium measurements for the glutamate-pyruvate transamination (3) were carried out at $I = 0.163$ with approximately 0.0123 M L-alanine. According to Smith & Smith (1937), γ for aqueous L-alanine is 1.00 at this concentration, but Joseph (1939) has shown that it is slightly decreased by the presence of salts; γ is therefore taken to be 0.95. For sodium pyruvate and sodium α -ketoglutarate γ is assumed to be the same as for sodium acetate and sodium fumarate, respectively, at the same total ionic strength. The respective values for the mean activity coefficients of these salts ($I = 0.163$) are 0.755 (Smith & Robinson, 1942) and 0.545 (Robinson *et al.* 1942). For sodium glutamate, the above extrapolation of the results of Hoskins *et al.* (1930) indicates that γ is equal to 0.61. The quotient of the activity coefficients is therefore

$$\begin{aligned} & \frac{\gamma_{\text{alanine}^{+-}} \times \gamma_{\alpha\text{-ketoglutarate}^{2-}}}{\gamma_{\text{glutamate}^{2-}} \times \gamma_{\text{pyruvate}^{-}}} \\ &= \frac{\gamma_{\text{alanine}^{+-}} \times (\gamma_{\text{disodium } \alpha\text{-ketoglutarate}})^2}{(\gamma_{\text{sodium glutamate}})^2 \times (\gamma_{\text{sodium pyruvate}})^2} \\ &= \frac{0.95 \times (0.545)^2}{(0.61)^2 \times (0.755)^2} = 0.73. \end{aligned}$$

Hence, for reaction (3)

$$\Delta G^{\circ} = -RT \ln (0.73 \times K_e) = -0.06 \text{ kcal.}$$

The data for reactions (2) and (3) indicate that

$$\Delta G^{\circ} = -1.15 \text{ kcal.}$$

for the reaction

A value is thus obtained for $\Delta G^{\circ}f$ of the pyruvate ion (-113.32 kcal.) using the values for alanine, oxaloacetate and aspartate. It is reasonably consistent with Blaschko's (1925) measurement of the heat of combustion of pyruvic acid and an assumed entropy of 54.7 (cf. Lipmann, 1941).

The values used for the activity coefficients of the glutamate and aspartate ions in the above calculation may have to be modified when more comprehensive activity data are available for sodium glutamate and aspartate, especially in the presence of other electrolytes. Nevertheless, it is unlikely that uncertainties in the activity coefficients introduce errors into the ΔG° values obtained for reactions (2) and (3)

of more than 0.05 and 0.15 kcal. respectively. These errors are not expected to be additive when reaction (4) is considered; the error from all causes in ΔG^0 for this reaction is probably less than 0.2 kcal.

Tricarboxylic acids. Starting from ΔG^0f for α -ketoglutarate, values may be obtained for isocitrate and oxalosuccinate using the free-energy changes for the dehydrogenation of isocitrate and its subsequent decarboxylation to α -ketoglutarate (cf. Kaplan, 1951). The ΔG^0 values used for these reactions are those calculated by Burton & Wilson (1953) from their value for ΔG^0 of reduction of triphosphopyridine nucleotide (TPN) and Ochoa's (1948) equilibrium measurements for the isocitric dehydrogenase and oxalosuccinic carboxylase systems. Since these measurements were performed at low ionic strengths (approx. 0.04) no correction is necessary for the activity coefficients. The instability of oxalosuccinate precluded accurate measurement of the equilibrium concentrations; hence the ΔG^0f value for this substance is less accurate than that for isocitrate.

ΔG^0f values for citrate and *cis*-aconitate are obtained from the isocitrate value using Krebs's (1953*a*) equilibrium data for the aconitase system. In evaluating ΔG^0 for the aconitase reactions, the activity coefficients of the trivalent anions have been assumed to be the same for the three tricarboxylic acids.

Lactate. ΔG^0f for the lactate ion (-123.64 kcal.) is obtained from the value for pyruvate and ΔG^0 for the dehydrogenation of lactate (Burton & Wilson, 1953). The available heat of combustion and entropy data (Parks & Huffman, 1932) give a ΔG^0f value of -123.74 kcal. for liquid D,L-lactic acid. This corresponds to about -121 kcal. for the aqueous L-lactate ion. The discrepancy of 2.5 kcal. between the two values for the lactate ion, is too large to be entirely explained by inaccuracies in the equilibrium constants. It is almost certainly due to an inaccuracy in the value used for the heat of combustion which is based on measurements of Emery & Benedict (1911) and of Meyerhof (1922). Many of the heats of combustion values obtained in this period have had to be revised by as much as 2 kcal./mole (cf. Rossini, 1932). The value of -123.64 kcal. obtained from equilibrium measurements is therefore adopted for the ΔG^0f of the lactate ion.

Acetoacetate and β -hydroxybutyrate. Approximate ΔG^0f values for these compounds may be obtained starting from the value for butyric acid (Table 1). Inspection of the ΔG^0f values for several compounds especially glycerol, *n*- and *iso*-propanol, malic acid and succinic acid suggest that the introduction of an oxygen atom into aqueous butyric acid to form β -hydroxybutyric acid involves a decrease of 36 ± 1 kcal. in the ΔG^0f . The value for aqueous β -hydroxybutyric acid is therefore about -127.5 kcal. and that for the anion about -121 kcal. Combining this value for β -hydroxybutyric with the potential measurements of Hoff-Jørgensen (1938) for the β -hydroxybutyric dehydrogenase reaction, ΔG^0f is found to be -115 kcal. for the acetoacetate ion. A similar value may be obtained on the assumption that the free-energy change of the reaction

is similar to those for the analogous carboxylations of pyruvate to oxaloacetate and of α -ketoglutarate to oxalosuccinate for which the $\Delta G'$ values are 7.50 and 6.88 kcal. respectively at pH 7. Taking the value of 7 kcal. for the carboxylation of acetone, ΔG^0f is -116 kcal. for acetoacetate.

The individual stages of the tricarboxylic acid cycle and associated reactions

Free-energy changes for the individual steps of the cycle and certain associated reactions are given in Table 5. With the exception of the values for the reactions involving the coenzyme of acetylation (CoA) they are derived from the data presented in the preceding sections of this paper. Since the conventional thermodynamic standard states are very different from the physiological conditions the free-energy changes are also given for the following concentrations of reactants: pH 7, 0.01M concentration of all dissolved reactants except the H^+ ion, 0.2 atm. oxygen and 0.05 atm. carbon dioxide. The free-energy changes computed for the concentrations of intermediates found by Frohman, Orten & Smith (1951) in rat tissues are very similar to those given in Table 5. Since not all of the free-energy data are accurate enough to justify the small corrections thus introduced it is preferable at this stage to give them for the arbitrary concentrations.

The free-energy changes of the reactions involving CoA were calculated with the aid of equilibrium data for the reaction

According to Stern, Shapiro, Stadtman & Ochoa (1951), K_c is about 5×10^5 at pH 7.4 (i.e. $\Delta G' = -7.8$ kcal.). The difference between this value and ΔG^0 for the condensation of oxaloacetate and acetate to form citrate (5.61 kcal.) shows that $\Delta G'$ is about 13.4 kcal. for the acetylation of CoA at pH 7.4. This is only an approximate value since neither the ΔG^0f value for acetic acid nor the equilibrium constant of reaction (5) possess the desired accuracy. Another value which is of the same order but probably no more reliable may be obtained using Stadtman's (1952) data for the reaction

K_c was found to be about 60 at pH 8; this corresponds to $\Delta G' = -2.4$ kcal. By analogy with the phosphoglyceric phosphokinase reaction (Bücher, 1947), ΔG^0 may be taken to be 4.5 kcal. for the reaction

The free energy of hydrolysis of ATP to ADP is evaluated in the next section of this paper and found to be -9.4 kcal. at pH 7.5. Hence $\Delta G'$ is about -13.9 kcal. for the hydrolysis of acetyl phosphate and -11.5 kcal. for the hydrolysis of acetyl CoA at pH 7.5.

Table 5. *Free-energy data for the steps of the tricarboxylic acid cycle and certain associated reactions*

(Temp., 25°; ΔG^0 values are for unit activity. ΔG values are for 0.2 atm. O_2 , 0.05 atm. CO_2 , pH 7 and 0.01 M concentration of other reactants.)

Reaction	ΔG (kcal.)	ΔG^0	
		(kcal.)	(kJ.)
Pyruvate ⁻ + $\frac{1}{2}O_2$ + CoA + H ⁺ → acetyl CoA + CO ₂	-55	-66	-15.8
Oxaloacetate ²⁻ + acetyl CoA + H ₂ O → citrate ³⁻ + CoA + H ⁺	-7.8	+1.8	+0.43
Oxaloacetate ²⁻ + acetate ⁻ → citrate ³⁻	+8.26	+5.53	1.32
Citrate ³⁻ → cis-aconitate ³⁻ + H ₂ O	+2.04	+2.04	0.488
cis-Aconitate ³⁻ + H ₂ O → isocitrate ³⁻	-0.45	-0.45	-0.167
isoCitrate ³⁻ + $\frac{1}{2}O_2$ → oxalosuccinate ³⁻ + H ₂ O	-52.5	-52.95	-12.66
Oxalosuccinate ³⁻ + H ⁺ → α-ketoglutarate ²⁻ + CO ₂	-8.6	-16.43	-3.93
Oxalosuccinate ³⁻ + H ₂ O → α-ketoglutarate ²⁻ + HCO ₃ ⁻	-9.5	-5.77	-1.38
α-Ketoglutarate ²⁻ + $\frac{1}{2}O_2$ → succinate ²⁻ + CO ₂	-69.8	-68.49	-16.37
Succinate ²⁻ + $\frac{1}{2}O_2$ → fumarate ²⁻ + H ₂ O	-35.7	-36.13	-8.63
Fumarate ²⁻ + H ₂ O → malate ²⁻	-0.88	-0.88	-0.21
Malate ²⁻ + $\frac{1}{2}O_2$ → oxaloacetate ²⁻ + H ₂ O	-44.8	-45.24	-10.81
$\frac{1}{2}$ Butyrate ⁻ + $\frac{1}{2}O_2$ + CoA + $\frac{1}{2}H^+$ → acetyl CoA + H ₂ O	-36	-42.5	-10.2
Acetate ⁻ + CoA + H ⁺ → acetyl CoA + H ₂ O	+16	+3.7	+0.9
2 Acetyl CoA + H ₂ O → acetoacetate + 2CoA + H ⁺	-13	-0.7	-0.17
Pyruvate ⁻ + 2 $\frac{1}{2}O_2$ + H ⁺ → 3CO ₂ + 2H ₂ O	-273.5	-282.84	-67.60
DPNH + $\frac{1}{2}O_2$ + H ⁺ → DPN ⁺ + H ₂ O	-51.9	-61.91	-14.80
Glucose + 6O ₂ → 6CO ₂ + 6H ₂ O	-688.5	-686.32	-164.03
Glutamate ²⁻ + $\frac{1}{2}O_2$ → α-ketoglutarate ²⁻ + NH ₄ ⁺	-45.9	-43.63	-1.04
Aspartate ²⁻ + $\frac{1}{2}O_2$ → oxaloacetate ²⁻ + NH ₄ ⁺	-44.7	-42.42	-1.01
Alanine ⁻ + $\frac{1}{2}O_2$ → pyruvate ⁻ + NH ₄ ⁺	-45.8	-43.57	-1.04
Glutamate ²⁻ + DPN ⁺ + H ₂ O → α-ketoglutarate ²⁻ + NH ₄ ⁺ + DPNH + H ⁺	+6.0	+18.28	4.37
Malate ²⁻ + TPN ⁺ → pyruvate ⁻ + TPNH + CO ₂	-2.0	-0.18	-0.04
Oxaloacetate ²⁻ + H ₂ O → pyruvate ⁻ + HCO ₃ ⁻	-9.12	-6.39	-1.53

The ΔG^0 value given in Table 5 (-43.63 kcal.) for the deamination of glutamate by reaction with molecular O_2 does not agree with that of -40.4 kcal. which may be calculated from the potential measurements of Filitti-Wurmser & Morel (1940). As discussed in the preceding paper (Burton & Wilson, 1953), there is often no guarantee that equilibrium has been reached in oxidation-reduction potential measurements in this type of system.

The ΔG^0 value for the reaction between glutamate and DPN corresponds to $K_a = 4.0 \times 10^{-14} M^2$. Olson & Anfinsen (1953) have obtained the value of $1.45 \times 10^{-13} M^2$ for K_c by direct measurement. It is likely that the activity coefficients account for a large part of the difference between the calculated K_a value and the experimental value for K_c .

In view of the high aconitase and fumarase activities of tissue homogenates it is interesting that Frohman *et al.* (1951) find that the tricarboxylic acids of rat tissues are present in ratios similar to those attained in the aconitase equilibrium, whereas the ratio of fumarate to malate found in the tissues (approx. 3:1) is very different to that of the fumarase equilibrium (approx. 0.2:1). This could be explained by assuming either that the fumarate and malate of the tissues are not in equilibrium or that most of the fumarate is present in a bound form.

SECTION 3

The free-energy changes associated with the hydrolysis of adenosinetriphosphate (ATP) to adenosinediphosphate (ADP)

Earlier evaluations of $\Delta G'$ for the hydrolysis of ATP to ADP and inorganic phosphate (Lipmann, 1941, 1946; Meyerhof, 1944; Dixon, 1949) have led to values which are close to -12 kcal. at pH 7.5. Despite the excellent agreement of these independent estimates, Dixon (1949), Meyerhof (1951) and Wurmser (1951) have each emphasized that some of the free-energy data on which they are based are very uncertain. Lately, Meyerhof himself has accepted a revised value of -10.5 kcal. (Oesper, 1951; Meyerhof, 1951), while Wurmser (1951) has suggested that the value of -9 kcal. is more consistent with the ΔG^0 values for glucose and lactate and equilibrium data for the stages of glycolysis. Although Wurmser's calculation is more reliable than the others mentioned, it is still based on uncertain free-energy data, the principal inaccuracies being in the thermochemical data for lactic acid and in Meyerhof & Green's (1949) ΔG^0 value for the hydrolysis of hexosediphosphate. These inaccuracies appear almost to cancel each other so that Wurmser's

value agrees with the independent value obtained below.

Evaluation of $\Delta G'$ for the hydrolysis of ATP. Most of the equilibria considered involve multivalent electrolytes, and cumbersome equations are therefore obtained when the ionization equilibria are taken into account. These may be conveniently avoided by considering the concentrations of those ionic forms which are the major component at pH 8. At this pH other ionic forms are present in negligible amounts. Under these conditions the hydrolysis of ATP may be formulated

By adding reactions (7)–(14) listed in Table 6 the following reaction is obtained

and the sum of the corresponding ΔG^0 values is 1.39 kcal. From this value and the ΔG^0_f values for aqueous glycerol (Table 1) and the pyruvate²⁻ ion (Table 4) the value of 1.03 kcal. is obtained for ΔG^0 of reaction (6), the hydrolysis of ATP to ADP. Since a H^+ ion appears on the right-hand side of equation (6) the relation between ΔG^0 and $\Delta G'$ (in kcal. at 25°) is

$$\Delta G' = \Delta G^0 - 1.3643 \times \text{pH},$$

which gives the value of -9.25 kcal. for $\Delta G'$ at pH 7.5. This value has been derived ignoring any possible effect of magnesium ions on the equilibria given in Table 6. Magnesium salts were added in the measurements of the phosphatase, phosphokinase, phosphoglyceric mutase and enolase equilibria (reactions 7, 12, 13 and 14) since they are required for full enzymic activity. It is necessary to consider whether the formation of complexes between the magnesium and the phosphorylated compounds is likely to affect the equilibrium measurements. This does not appear to have been tested experimentally and, unfortunately, most of the information about the formation of complexes between Mg^{2+} ions and phosphate compounds is only qualitative (Frankenthal, 1944; Neuberg & Mandl, 1949; Hers, 1952). Quantitative data based on potentiometric titration are available for inorganic phosphate; the results of Greenwald, Redish & Kibrick (1940) indicate that Mg^{2+} and HPO_4^{2-} ions form a complex, probably MgHPO_4 , and that the dissociation constant is

$$\begin{aligned} K' &= [\text{Mg}^{2+}][\text{HPO}_4^{2-}]/[\text{MgHPO}_4] \\ &= \text{antilog}_{10}(2.15 \sqrt{I} - 2.50). \end{aligned}$$

To obtain quantitative information for ATP and ADP, 0.004–0.018 M solutions of the sodium salts have been titrated with 0.5 N-HCl: the pH was measured between 8 and 2 with a glass electrode (Burton, unpublished). In the presence of increasing concentrations of MgCl_2 , the apparent pK of the weakly ionizing phosphate group becomes more acidic while the pK of the adenine amino group is unchanged, the effect being greater with ATP than with ADP. The results are compatible with the formulation of the principal complexes as MgATP^{2-} and MgADP^{2-} with dissociation constants of about 1.2×10^{-3} and 2.7×10^{-3} M respectively at an ionic strength of 0.2. Similar measurements on the Mg complex of phosphopyruvate give a dissociation constant of 10^{-3} M. For the same ionic strength the results of Greenwald *et al.* (1940) give a constant of 3×10^{-3} M for the complex of

Table 6. *Thermodynamic data for the conversion of glycerol to pyruvate*

(Addition of ΔG^0 for reactions (7)–(14) gives ΔG^0 for reaction (15). This value is used in the text to calculate ΔG^0 for the hydrolysis of ATP.)

Reaction	K_c	ΔG^0 (kcal. at 25°)	Source
(7) Glycerol + HPO_4^{2-} → DL-glycerol 1-phosphate ²⁻ + H_2O	0.0122 (37°)	2.24	Meyerhof & Green (1949)
(8) DL-Glycerol 1-phosphate ²⁻ → L-glycerol 1-phosphate ²⁻	—	0.41	Calculated (= $RT \ln 2$)
(9) L-Glycerol 1-phosphate ²⁻ → dihydroxyacetone phosphate ²⁻ + H_3	—	10.24	Burton & Wilson (1953)
(10) Dihydroxyacetone phosphate ²⁻ → glyceraldehyde 3-phosphate ²⁻ + H_3	0.045	1.83	Meyerhof & Junowicz-Kocholaty (1943)
(11) Glyceraldehyde 3-phosphate ²⁻ + HPO_4^{2-} → glyceroylphosphate 3-phosphate ⁴⁻ + H_3	—	5.83	From data of Meyerhof & Oesper (1947) see Burton & Wilson (1953)
(12) Glyceroylphosphate 3-phosphate ⁴⁻ + ADP^{3-} → glycerate 3-phosphate ³⁻ + ATP^{4-}	3200	-4.78	Bücher (1947)
(13) Glycerate 3-phosphate ³⁻ → enolpyruvate 2-phosphate ³⁻ + H^+	—	0.42	Meyerhof & Oesper (1949)
(14) Enolpyruvate 2-phosphate ³⁻ + ATP^{4-} → pyruvate ²⁻ + ATP^{4-}	7.1×10^{10}	-14.80	—
(15) Glycerol + 2HPO_4^{2-} + 2ADP^{3-} + H^+ → pyruvate ²⁻ + 2ATP^{4-} + $2\text{H}_2\text{O}$ + 2H_3	—	1.39	—

inorganic phosphate. For present purposes it is assumed that phosphate compounds not containing the pyrophosphate group possess an affinity for Mg^{2+} ions similar to that of phosphopyruvate. The above dissociation constants then indicate that, in Bücher's (1947) measurements of the phosphoglyceric phosphokinase equilibrium, 10–15 % of the ATP and 5–10 % of the ADP were present as Mg complexes. In the case of the pyruvate phosphokinase equilibrium (Meyerhof & Oesper, 1949) it appears that about 30 % of the ATP and 20 % of the ADP were present as complexes. The corrections thus introduced into the ΔG^0 values for these reactions are very small and correspond to an increase of about 0.1 kcal. in $\Delta G'$ for the hydrolysis of ATP. The value of 9.4 kcal. at pH 7.5 is therefore adopted for subsequent calculations.

The largest single source of error in this value is probably ΔG^0 for the pyruvate phosphokinase reaction (14) since the pH was not reported in the only equilibrium measurements which are available (Meyerhof & Oesper, 1949). In a private communication Dr Meyerhof has stated that the pH was probably about 7.6. The data for the phosphoglyceric mutase and enolase equilibria (13) may introduce a small additional error because the equilibrium constant obtained in the presence of crystalline enolase (Warburg & Christian, 1942) does not agree with that calculated by Meyerhof & Oesper (1949) from the equilibrium in the presence of a crude enzyme system containing phosphoglyceric mutase together with data for the phosphoglyceric mutase equilibrium. The discrepancy amounts to a factor of 2.05 in the enolase equilibrium (0.43 kcal.). The values of Meyerhof & Oesper have been adopted in Table 6 because they are supported by earlier measurements (Lohmann & Meyerhof, 1934; Meyerhof & Kiessling, 1935; Akano, 1935). The equilibrium data for the other reactions in Table 6 are based on extensive measurements mainly with purified enzyme systems, and in most instances they have been confirmed by independent investigators. It should be pointed out that only half of any error in one of these ΔG^0 values is introduced into the value of $\Delta G'$ for the hydrolysis of ATP.

The ΔG^0 values for the dehydrogenation of glycerol phosphate and triosephosphate were calculated from data at low ionic strengths where the activity coefficients are presumably close to unity. Activity data are not available for the other reactions of Table 6 but, with the possible exception of the pyruvate phosphokinase reaction, it is probable that the activity coefficients of substances on opposite sides of each reaction will largely cancel each other.

The value of -9.4 kcal. for the free energy of hydrolysis of ATP at pH 7.5 refers to thermodynamic standard conditions (i.e. molal concentrations of ATP, ADP and inorganic phosphate). At the concentrations usually present in tissue homogenate experiments and in intact cells the numerical value of ΔG is greater. The relation between ΔG (in kcal. at 25°) and ΔG^0 is as follows

$$\Delta G = \Delta G^0 - 1.3643 \times \left\{ pH + \log_{10} \frac{[ATP^4-]}{[ADP^3-][HPO_4^{2-}]} \right\}.$$

Between pH 7.5 and 11 the concentration of each of these ions is practically equal to the total concentration of the respective compound. There is therefore in this range a linear relationship between ΔG

and pH for constant concentrations of ATP, ADP and inorganic phosphate, although in more acid solutions other ionic forms may predominate with the result that, as discussed by Dixon (1949) and by Alberty, Smith & Bock (1951) ΔG becomes practically independent of the pH below pH 6.3.

Homogenate experiments. The concentrations used in the homogenate experiments of Krebs, Ruffo, Johnson, Eggleston & Hems (1953) are of the same order as those in many previous studies of oxidative phosphorylation and are as follows: $5 \times 10^{-3} M$ inorganic phosphate, $2.5 \times 10^{-3} M$ ATP, about $2 \times 10^{-4} M$ ADP and $2.5 \times 10^{-3} M$ $MgCl_2$ at pH 7.4. Under these conditions ΔG is calculated to be -14 kcal. ignoring the complex-forming effect of the Mg^{2+} ion and -13.8 kcal. when this is taken into account.

Animal tissues. ΔG may be calculated for the concentrations of ATP, ADP and inorganic phosphate reported to be present in animal tissues. Taking the pH to be 7, the data of LePage (1946) for rat tissues (as given by Dickens, 1951) give values for ΔG which are between -12.5 and -16 kcal. neglecting any complex formation between magnesium and the phosphate compounds. If the intracellular pH is 6.3 the values would be about 0.5 kcal. more positive. These are only rough estimates of the free energy changes since they are calculated from relatively few analyses on the assumption that the ATP, ADP and inorganic phosphate are uniformly distributed throughout the tissues. It might be thought that the tissue magnesium ($3\text{--}4 \mu\text{moles/g.}$) could as a result of complex formation invalidate these results, but since all three reactants are likely to form complexes the corrections to be applied for this effect amount to less than 0.3 kcal. in the ΔG values.

Hydrolysis of ADP and creatinephosphate. Other high-energy phosphate compounds in tissues are likely to be almost in equilibrium with ATP and ADP as the activities of the phosphokinases are relatively high. Hence the ΔG values for the hydrolysis of creatinephosphate and the terminal phosphate group of ADP in tissues are likely to be close to that for the hydrolysis of the terminal phosphate group of ATP even if the free-energy changes at standard concentrations (ΔG^0) may not be the same.

Measurements of the myokinase equilibrium (Eggleston & Hems, 1952) show that slightly more free energy is liberated by the hydrolysis of ADP to AMP under standard conditions than by the hydrolysis of ATP to ADP. The difference amounts to 0.48 kcal. ignoring the effect of Mg^{2+} ions on the equilibrium; it is expected to be 0.6–0.8 kcal. if the Mg^{2+} ion effect is taken into account.

Banga's (1943) measurements of the creatine phosphokinase equilibrium when combined with the

above value of $\Delta G'$ for the hydrolysis of ATP indicate that ΔG^0 is about -12.8 kcal. for the hydrolysis of creatinephosphate in the pH range between 7.5 and 11

SECTION 4

The free-energy changes associated with the individual steps of glycolysis and alcoholic fermentation

Equilibrium measurements (including those referred to in Table 6) are available for all the individual enzyme-catalysed reactions of glycolysis except the glucokinase and phosphofructokinase reactions. The ΔG^0 values for these reactions may be calculated by combining the value for the hydrolysis of ATP with those for the hydrolysis of the hexose phosphates. Although the thermochemical data for starch and glycogen are inadequate, ΔG^0 for the glucose units of these polysaccharides may be evaluated using data for glucose 6-phosphate and for the phosphoglucumutase and phosphorylase reactions.

ΔG^0 for the hydrolysis of hexose phosphates. Meyerhof & Green (1949) have measured the equilibria between several sugar phosphates, the free sugar and inorganic phosphate in the presence of purified intestinal phosphatase. The equilibria are very much in favour of hydrolysis and high concentrations of the sugar and of inorganic phosphate were necessary. Inadequate data are available to evaluate the thermodynamic activities under these conditions so that the ΔG^0 values are not as reliable as most values derived from equilibrium measurements. The ΔG^0 values for glucose 6-phosphate (G 6-P) and fructose 6-phosphate (F 6-P) are -3.0 and -3.3 kcal. respectively. Slein's (1950) measurements of the phosphoglucumutase equilibrium give the value of 0.50 kcal. for ΔG^0 of the conversion of G 6-P to F 6-P and Wolfrom & Lewis (1928) found that, in the epimerization of glucose, the ratio of fructose to glucose became constant when it reached 0.49 ; if this ratio represents the equilibrium between glucose and fructose, ΔG^0 is 0.44 kcal. The difference of 0.06 kcal. between the ΔG^0 value for the epimerization of glucose and that for the phosphoglucumutase reaction should equal the difference between the ΔG^0 value for the hydrolysis of F 6-P and that for G 6-P. There is therefore a small discrepancy in these data, but the evidence is inadequate to decide whether Meyerhof & Green's (1949) values are at fault or whether the ΔG^0 value for the epimerization is incorrect and somewhat arbitrary values must therefore be adopted. In this paper the ΔG^0 values of -3.1 and -3.2 kcal. are used for the hydrolysis of G 6-P and F 6-P respectively.

Meyerhof & Green's ΔG^0 values for the hydrolysis of fructose 1:6-diphosphate (HDP) cannot be accepted on the present evidence since the F 6-P and fructose 1-phosphate (F 1-P) in the equilibrium mixture probably interfered in the estimation of HDP by conversion to triosephosphate under the action of aldolase. The available evidence indicates that aldolase is not specific to HDP and may act on F 6-P (Dounce, Barnett & Beyer 1950), and possibly also on F 1-P since Meyerhof, Lohmann & Schuster (1936)

have shown that dihydroxyacetone phosphate may condense with D-glyceraldehyde to form F 1-P, and it is therefore likely that the reverse reaction can occur. The following alternative procedure for the evaluation of ΔG^0 for the hydrolysis of HDP gives a value (-7.6 kcal.) which differs from that of Meyerhof & Green (-4.8 kcal.) in the expected direction.

From Meyerhof & Lohmann's (1934) data for the equilibrium between dihydroxyacetone phosphate and HDP under the action of aldolase and isomerase, ΔG^0 is -3.68 kcal. at 25° . The reaction is formulated

and on adding to it twice the sum of the reactions (7)–(9) of Table 6, the following reaction is obtained

Addition of the corresponding ΔG^0 values gives the value of 22.10 kcal. for reaction (17). From this value, the ΔG^0 value for the conversion of glucose to fructose (0.44 kcal. from Wolfrom & Lewis's data), and from the ΔG^0 values for glucose and glycerol (Table 1) ΔG^0 is found to be -7.6 kcal. for the reaction

Starch and glycogen. In the synthesis or degradation of either starch or glycogen under the influence of the appropriate phosphorylase, the reaction ceases when the ratio of the concentrations $[\text{G 1-P}^{2-}]/[\text{HPO}_4^{2-}]$ becomes 0.40 (Hanes & Maskell, 1942; Cori, Cori & Green, 1943). The reaction may be formulated

The equilibrium ratio of G 1-P to inorganic phosphate is independent of the polysaccharide concentration. This is expected if, as is likely, all the glucose units of the polysaccharide which react in the phosphorylase system are associated with the same free energy. ΔG^0 for the hydrolysis of G 1-P (-4.8 kcal.) may be obtained from the value for G 6-P and the phosphoglucumutase equilibrium (Colowick & Sutherland, 1942). The free-energy change for the hydrolysis of a glycosidic link of starch or glycogen is therefore $-4.8 - RT \ln 0.40 = -4.3$ kcal. This, together with the ΔG^0 value for glucose, gives a ΔG^0 value of -158.4 kcal. for the glucose units ($\text{C}_6\text{H}_{10}\text{O}_5$) of the polysaccharides.

It should be emphasized that this value refers only to those glucose units which react in the phosphorylase system and, like the equilibrium of that system, is independent of the polysaccharide concentration.

The individual reactions of glycolysis and alcoholic fermentation. The free-energy data for the stages of these processes (Table 7) are based on the ΔG^0 values in Table 1 for glucose, ethanol, acetaldehyde and carbon dioxide, on data for the hydrolysis of ATP to ADP, on some data given in Table 6, and on the equilibrium measurements of Racker (1950) for the lactic and alcohol dehydrogenase systems, together with the data already presented in this section.

It will be seen that the conversion of glycogen to lactate liberates about 28 kcal./mole lactate $^-$ ion at pH 7. According to the accepted mechanism of glycolysis (cf. Dickens, 1951) this is accompanied by

Table 7. Free-energy data for the steps of glycolysis and alcoholic fermentation

(Temp., 25°; ΔG^0 values are for unit activity, ΔG values are for 0.2 atm. O_2 ; 0.05 atm. CO_2 ; pH 7 and 0.01 M concentration of other reactants except glycogen. As discussed in the text, the data for the reactions involving glycogen are independent of the concentration of glycogen.)

	Reaction	ΔG (kcal.)	ΔG^0	
			(kcal.)	kJ.
Glycogen (1 glucose unit) + H_2O	$\rightarrow 2 \text{ lactate}^- + 2H^+$	-56.7	-32.2	-7.7
Glucose	$\rightarrow 2 \text{ lactate}^- + 2H^+$	-49.7	-27.9	-6.67
Glucose	$\rightarrow 2 \text{ ethanol} + 2CO_2$	-62.2	-55.9	-13.36
Glucose + ATP^{4-}	$\rightarrow G 6-P^{2-} + ADP^{3-} + H^+$	-5.7	+3.9	+0.93
G 6- P^{2-}	$\rightarrow G 1-P^{2-}$	+1.72	+1.72	+0.41
G 1- P^{2-} + H_2O	$\rightarrow \text{glycogen} + HPO_4^{2-}$	-0.55	-0.55	-0.13
G 6- P^{2-}	$\rightarrow F 6-P^{2-}$	+0.50	+0.50	+0.12
F 6- P^{2-} + ATP^{4-}	$\rightarrow HDP^{4-} + ADP^{3-} + H^+$	-4.3	+5.2	+1.24
HDP $^{4-}$	$\rightarrow \text{glyceraldehyde } 3-P^{2-} + \text{dihydroxyacetone } P^{2-}$	+2.78	+5.51	+1.32
Dihydroxyacetone- P^{2-}	$\rightarrow \text{glyceraldehyde } 3-P^{2-}$	+1.83	+1.83	+0.44
Glyceraldehyde 3- P^{2-} + $DPN^+ + HPO_4^{2-}$	$\rightarrow \text{glyceroyl-P } 3-P^{4-} + DPNH + H^+$	+4.2	+11.05	+2.64
Glyceroyl-P 3- P^{4-} + ADP^{3-}	$\rightarrow \text{glycerate } 3-P^{3-} + ATP^{4-}$	-4.75	-4.75	-1.14
Glycerate 3- P^{3-}	$\rightarrow \text{glycerate } 2-P^{3-}$	+1.06	+1.06	+0.25
Glycerate 2- P^{3-}	$\rightarrow \text{enolpyruvate } 2-P^{3-} + H_2O$	-0.64	-0.64	-0.15
Enolpyruvate 2- P^{3-} + $ADP^{3-} + H^+$	$\rightarrow \text{pyruvate}^- + ATP^{4-}$	-5.0	-14.5	-3.46
Pyruvate $^-$ + $DPNH + H^+$	$\rightarrow \text{lactate}^- + DPN^+$	-6.0	-15.54	-3.71
Pyruvate $^-$ + H^+	$\rightarrow \text{acetaldehyde} + CO_2$	-6.9	-14.65	-3.50
Acetaldehyde + $DPNH + H^+$	$\rightarrow \text{ethanol} + DPN^+$	-5.4	-14.90	-3.56
$ATP^{4-} + H_2O$	$\rightarrow ADP^{3-} + HPO_4^{2-} + H^+$	-11.3	+0.9	+0.21

a net formation of 1.5 moles high-energy phosphate ($\sim P$) per mole of lactate formed. Assuming 14 kcal. per mole $\sim P$ this would require 21 kcal. or 75 % of the total free energy available. If glucose is the starting material, about 25 kcal. are available per mole of lactate, but the net amount of $\sim P$ formed is 1 mole per mole lactate, which corresponds to about 56 % of the total free energy available. The free energy available from the alcoholic fermentation of glucose (31 kcal./mole ethanol) is appreciably greater than that from the lactic fermentation; the formation of 1 $\sim P$ per mole ethanol corresponding to 14 kcal. represents 44 % of the available energy.

SUMMARY

1. The free-energy changes for the individual stages of the main biological energy-giving reactions have been re-evaluated with improved accuracy.

2. Data for the heats of combustion and entropies of compounds of biological interest have been critically selected and used to evaluate the free energies of formation (ΔG^0f) which are listed in Table 1.

3. From values contained in Table 1 and equilibrium data for reactions associated with the tricarboxylic acid cycle values of ΔG^0f (listed in Table 4) have been obtained for several compounds which are intermediates of, or associated with, the cycle. The free-energy changes for the stages of the tricarboxylic acid cycle and for certain associated reactions are given in Table 5 and those for the stages of glycolysis and alcoholic fermentation are given in Table 7.

4. The free-energy change associated with the hydrolysis of adenosinetriphosphate (ATP) to adenosinediphosphate (ADP) and inorganic phosphate has been evaluated from the values of ΔG^0f for glycerol and pyruvate and from the equilibrium data for the enzymic stages of the conversion of glycerol to triosephosphate, and subsequently to pyruvate, coupled with the phosphorylation of ADP. The value obtained is -9.4 kcal. for pH 7.5 and standard concentrations (1M activity) of ATP, ADP and inorganic phosphate; for the concentrations in animal tissues it is 13-16 kcal.

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REFERENCES

- Ahlberg, J. E., Blanchard, E. R. & Lundberg, W. O. (1937). *J. Chem. Phys.* **5**, 539.
- Akano, R. (1935). *Biochem. Z.* **280**, 110.
- Alberty, R. A., Smith, R. M. & Bock, R. M. (1951). *J. biol. Chem.* **193**, 425.
- Anderson, A. G. & Stegeman, G. (1941). *J. Amer. chem. Soc.* **63**, 2119.
- Banga, I. (1943). *Stud. Inst. med. Chem. Univ. Szeged.* **3**, 59. Cited in *Chem. Abstr.* (1947), **41**, 1301.
- Batchelder, A. C. & Schmidt, C. L. A. (1940). *J. phys. Chem.* **44**, 893.
- Berkeley, Earl of & Hartley, E. G. J. (1906). *Phil. Trans. A*, **206**, 481.
- Bichowsky, F. R. & Rossini, F. D. (1936). *Thermochemistry of the Chemical Substances*. New York: Reinhold.
- Blaschko, H. (1925). *Biochem. Z.* **158**, 428.
- Borsook, H. & Huffman, H. M. (1938). In *Chemistry of the Amino Acids and Proteins*, ed. by Schmidt, C. L. A., Springfield: Charles C. Thomas.
- Borsook, H. & Schott, H. F. (1931). *J. biol. Chem.* **92**, 535.
- Bücher, T. (1947). *Biochim. biophys. Acta*, **1**, 292.
- Burton, K. (1952). *Biochim. biophys. Acta*, **8**, 114.
- Burton, K. & Wilson, T. H. (1953). *Biochem. J.* **54**, 86.
- Butler, J. A. V., Ramchandani, C. N. & Thomson, D. W. (1935). *J. chem. Soc.* p. 280.
- Clarke, T. H. & Stegeman, G. (1939). *J. Amer. chem. Soc.* **61**, 1726.
- Colowick, S. P. & Sutherland, E. W. (1942). *J. biol. Chem.* **144**, 423.
- Cori, C. F., Cori, G. T. & Green, A. A. (1943). *J. biol. Chem.* **151**, 39.
- Darling, S. (1945). *Acta physiol. scand.* **10**, 150.
- Darling, S. (1947). *Nature, Lond.*, **160**, 403.
- Daudt, W. (1923). *Z. phys. Chem.* **106**, 255.
- Dehn, W. M. (1917). *J. Amer. chem. Soc.* **39**, 1399.
- Dickens, F. (1951). In *The Enzymes*, ed. by Sumner, J. B. & Myrback, K. New York: Academic Press Inc.
- Dixon, M. (1949). *Multi-Enzyme Systems*, Cambridge University Press.
- Doudoroff, M. (1943). *J. biol. Chem.* **151**, 351.
- Dounce, A. L., Barnett, S. R. & Beyer, G. T. (1950). *J. biol. Chem.* **185**, 769.
- Drucker, K. & Moles, E. (1909). *Z. phys. Chem.* **75**, 405.
- Dulitskaya, K. A. (1945). *J. gen. Chem., Moscow*, **15**, 9.
- Eggleson, L. V. & Hems, R. (1952). *Biochem. J.* **52**, 156.
- Emery, A. G. & Benedict, F. G. (1911). *Amer. J. Physiol.* **28**, 301.
- Filitti-Wurmser, S. & Morel, M. (1940). *Enzymologia*, **9**, 369.
- Frankenthal, L. (1944). *J. Amer. chem. Soc.* **66**, 2124.
- Fredenhagen, K. & Liebster, H. (1932). *Z. phys. Chem. A*, **162**, 449.
- Fricke, R. (1929). *Z. Electrochem.* **35**, 631.
- Frohmman, C. E., Orten, J. M. & Smith, A. H. (1951). *J. biol. Chem.* **193**, 277.
- Greenwald, I., Redish, J. & Kibrick, A. C. (1940). *J. biol. Chem.* **135**, 65.
- Grover, D. W. & Nicol, J. M. (1940). *J. Soc. chem. Ind., Lond.*, **59**, 175.
- Halford, J. O. (1941). *J. chem. Phys.* **9**, 859.
- Hanes, C. S. & Maskell, E. J. (1942). *Biochem. J.* **36**, 76.
- Harned, H. S. & Davis, R. (1943). *J. Amer. chem. Soc.* **65**, 2030.
- Hendricks, B. C., Dorsey, J. H., LeRoy, R. H. & Moseley, A. G. (1930). *J. phys. Chem.* **34**, 418.
- Hendricks, B. C., Steinbach, W. A., LeRoy, R. H. & Moseley, A. G. (1934). *J. Amer. chem. Soc.* **56**, 99.
- Hers, H. G. (1952). *Biochim. biophys. Acta*, **8**, 424.
- Higbie, H. & Stegeman, G. (1950). *J. Amer. chem. Soc.* **72**, 3799.
- Hoff-Jørgensen, E. (1938). *Skand. Arch. Physiol.* **80**, 176.
- Hoskins, W. M., Randall, M. & Schmidt, C. L. A. (1930). *J. biol. Chem.* **88**, 215.
- Hudson, C. S. (1908). *J. Amer. chem. Soc.* **30**, 1767.
- Huffman, H. M. (1942). *J. phys. Chem.* **46**, 885.
- Huffman, H. M. & Fox, S. W. (1938). *J. Amer. chem. Soc.* **60**, 1400.
- Jack, G. W. & Stegeman, G. (1941). *J. Amer. chem. Soc.* **63**, 2121.
- Jackson, R. F. (1917). *Bull. U.S. Bur. Stand.* **13**, 633.
- Jackson, R. F. & Silsbee, C. G. (1922). *Bull. U.S. Bur. Stand.* **17**, 715.
- Joseph, N. R. (1939). *J. biol. Chem.* **130**, 203.
- Kalokar, H. M. (1941). *Chem. Rev.* **28**, 71.
- Kaplan, N. O. (1951). In *The Enzymes*, ed. by Sumner, J. B. & Myrback, K. New York: Academic Press Inc.
- Krebs, H. A. (1953a). *Biochem. J.* **54**, 78.
- Krebs, H. A. (1953b). *Biochem. J.* **54**, 82.
- Krebs, H. A., Ruffo, A., Johnson, M., Eggleston, L. V. & Hems, R. (1953). *Biochem. J.* **54**, 107.
- LePage, G. A. (1946). *Amer. J. Physiol.* **147**, 446.
- Lewis, G. N. & Randall, M. (1923). *Thermodynamics*, 1st ed. New York: McGraw-Hill Book Co. Inc.
- Lipmann, F. (1941). *Advanc. Enzymol.* **1**, 99.
- Lipmann, F. (1946). *Advanc. Enzymol.* **6**, 231.
- Lohmann, K. & Meyerhof, O. (1934). *Biochem. Z.* **273**, 60.
- Meyerhof, O. (1922). *Biochem. Z.* **129**, 594.
- Meyerhof, O. (1944). *Ann. N.Y. Acad. Sci.* **45**, 377.
- Meyerhof, O. (1951). In *Phosphorus Metabolism*, ed. by McElroy, W. D. & Glass, B. Baltimore: John Hopkins.
- Meyerhof, O. & Green, H. (1949). *J. biol. Chem.* **178**, 655.
- Meyerhof, O. & Kiessling, W. (1935). *Biochem. Z.* **280**, 99.
- Meyerhof, O. & Junowicz-Kocholat, R. (1943). *J. biol. Chem.* **149**, 71.
- Meyerhof, O. & Lohmann, K. (1934). *Biochem. Z.* **271**, 89.
- Meyerhof, O., Lohmann, K. & Schuster, P. (1936). *Biochem. Z.* **286**, 319.
- Meyerhof, O. & Oesper, P. (1947). *J. biol. Chem.* **170**, 1.
- Meyerhof, O. & Oesper, P. (1949). *J. biol. Chem.* **179**, 1371.
- Monod, J. & Torriani, A. M. (1950). *Ann. Inst. Pasteur*, **78**, 65.
- Neuberg, C. & Mandl, I. (1949). *Arch. Biochem.* **23**, 499.
- Neuberg, A. (1936). *Biochem. J.* **30**, 2085.
- Ochoa, S. (1948). *J. biol. Chem.* **174**, 133.
- Ochoa, S. (1951). *Physiol. Rev.* **31**, 56.
- Oesper, P. (1951). In *Phosphorus Metabolism*, ed. by McElroy, W. D. & Glass, B. Baltimore: John Hopkins.
- Ogston, A. G. (1951). Report to 8th Conseil de Chimie Solvay. Cited by Wurmser, R. (1951), *Ann. Rev. Biochem.* **20**, 1.
- Ogston, A. G. & Smithies, O. (1948). *Physiol. Rev.* **28**, 283.

- Olson, J. A. & Anfinsen, C. B. (1953). *J. biol. Chem.* (in the Press).
- Parks, G. S. & Huffman, H. M. (1932). *The Free Energies of Some Organic Compounds*. New York: Reinhold.
- Parks, G. S., Huffman, H. M. & Barmore, M. (1933). *J. Amer. chem. Soc.* **55**, 2733.
- Parks, G. S., West, T. J., Naylor, B. F., Fujii, P. S. & McClaine, L. A. (1946). *J. Amer. chem. Soc.* **68**, 2524.
- Pinching, G. D. & Bates, R. G. (1950). *J. Res. nat. Bur. Stand.* **45**, 322, 444.
- Racker, E. (1950). *J. biol. Chem.* **184**, 313.
- Robinson, R. A., Smith, P. K. & Smith, E. R. B. (1942). *Trans. Faraday Soc.* **38**, 63.
- Rossini, F. D. (1932). *J. Res. nat. Bur. Stand.* **8**, 119.
- Rossini, F. D. (1934). *J. Res. nat. Bur. Stand.* **13**, 189.
- Rossini, F. D., Wagman, D. D., Evans, W. H., Levine, S. & Jaffe, I. (1952). *Selected Values of Chemical Thermodynamic Properties*. Nat. Bur. Stand. Circular no. 500.
- Saxton, B. & Darken, L. S. (1940). *J. Amer. chem. Soc.* **62**, 846.
- Scatchard, G., Hamer, W. J. & Wood, S. E. (1938). *J. Amer. chem. Soc.* **60**, 3061.
- Schmidt, C. L. A. (1938). *Chemistry of the Amino Acids and Proteins*. Springfield: Charles C. Thomas.
- Slein, M. W. (1950). *J. biol. Chem.* **186**, 753.
- Smith, E. R. B. & Smith, P. K. (1942). *J. biol. Chem.* **146**, 187.
- Smith, P. K. & Smith, E. R. B. (1937). *J. biol. Chem.* **121**, 607.
- Smith, E. R. B. & Robinson, R. A. (1942). *Trans. Faraday Soc.* **38**, 70.
- Stadtman, E. R. (1952). *J. biol. Chem.* **196**, 535.
- Stedman, D. F. (1928). *Trans. Faraday Soc.* **24**, 289.
- Stern, J. R., Shapiro, B., Stadtman, E. R. & Ochoa, S. (1951). *J. biol. Chem.* **193**, 703.
- Stiehler, R. D. & Huffman, H. M. (1935). *J. Amer. chem. Soc.* **57**, 1734, 1741.
- Sturtevant, J. M. (1937). *J. Amer. chem. Soc.* **59**, 1528.
- Warburg, O. & Christian, W. (1942). *Biochem. Z.* **310**, 384.
- Wolfom, M. L. & Lewis, W. L. (1928). *J. Amer. chem. Soc.* **50**, 837.
- Woods, D. D. (1936). *Biochem. J.* **30**, 515.
- Woolf, B. (1929). *Biochem. J.* **23**, 472.
- Wurmser, R. (1951). *Ann. Rev. Biochem.* **20**, 1.

Oxidative Phosphorylation

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It is known from the work of Engelhardt (1930, 1932), Runnström, Lennerstrand & Borei (1934), Kalckar (1939, 1941, 1944), Belitzer & Tsibakowa (1939), Cori and his associates (Colowick, Welch & Cori, 1940; Colowick, Kalckar & Cori, 1941) and Ochoa (1941, 1943, 1947) that the free energy of oxidative processes can be used for the synthesis of organic phosphates, including those containing energy-rich bonds, namely, adenosinetriphosphate (ATP) or creatine phosphate. The quantitative aspects of the 'oxidative phosphorylation' i.e. the value of the ratio

$$\frac{\text{equivalents of organic phosphate formed}}{\text{atoms of oxygen consumed}}$$

has been studied by Ochoa (1941, 1943) and more recently by Hunter & Hixon (1949*a, b*), Lehninger & Smith (1949), Green, Atchley, Nordmann & Teply (1949), Pardee & Potter (1949), Slater (1950), Judah & Williams-Ashman (1951) and others. The values obtained for the above ratio, henceforth in this paper called 'phosphorylation quotient', depend on experimental conditions. Of special interest are the maximum values that can be

obtained. The highest quotients found so far approach the value of 4 (Barkulis & Lehninger, 1951*a*; Judah, 1951). These values were usually obtained with tissue preparations which contained, apart from the oxidizing enzymes, ATP, hexokinase, inorganic phosphate and fluoride, and under these conditions most of the inorganic phosphate utilized appears as hexosephosphate. The presence of fluoride is required to prevent dephosphorylation of organic phosphate. The phosphate uptake values measured by this procedure have to be regarded as minimum values as dephosphorylation may not be completely abolished.

The present paper is concerned with measurements of the phosphorylation quotient by a procedure employing ^{32}P which has the advantage of being independent of the action of dephosphorylating enzymes. The use of ^{32}P as a tracer, combined with newer methods of separating the phosphorylated compounds by paper chromatography, makes it possible to measure directly the rate of synthesis of ATP even when no changes in the concentrations of ATP or of inorganic phosphate occur. It is therefore unnecessary to add fluoride or to apply corrections.