SUMMARY

1. The succinic oxidase system was reconstructed, in presence of exogenous cytochrome *c*, from two inactive preparations, one, from the *Neurospora* strain *C117*, providing succinic dehydrogenase, the other from alkali-treated wild-type *Neurospora* or heart muscle preparations, providing cytochrome oxidase.

2. It was found in spectroscopic experiments that particles from the *Neurospora* strain *poky* reacted directly with wild-type or heart muscle particles to reconstruct the succinic oxidase system in the absence of a soluble component.

I wish to thank Professor D. Keilin, F.R.S., for his interest and advice, and Mr P. B. Scutt for the samples of heart muscle preparation.

REFERENCES

- Cleland, K. W. & Slater, E. C. (1953). *Biochem. J.* **53**, 547.
 Haskins, F. A., Tissières, A., Mitchell, H. K. & Mitchell, M. B. (1953). *J. biol. Chem.* **200**, 819.
 Keilin, D. & Hartree, E. F. (1940). *Proc. Roy. Soc. B*, **129**, 277.
 Keilin, D. & Hartree, E. F. (1946). *Nature, Lond.*, **157**, 210.
 Keilin, D. & Hartree, E. F. (1947). *Biochem. J.* **41**, 500.
 Mitchell, M. B. & Mitchell, H. K. (1952). *Proc. nat. Acad. Sci., Wash.*, **38**, 442.
 Mitchell, M. B., Mitchell, H. K. & Tissières, A. (1953). *Proc. nat. Acad. Sci., Wash.*, **39**, 606.
 Slater, E. C. (1949). *Biochem. J.* **45**, 1.
 Tissières, A. & Mitchell, H. K. (1954). *J. biol. Chem.* **208**, 241.

Thermodynamic Quantities for the Dissociation Equilibria of Biologically Important Compounds

3. THE DISSOCIATIONS OF THE MAGNESIUM SALTS OF PHOSPHORIC ACID, GLUCOSE 1-PHOSPHORIC ACID AND GLYCEROL 2-PHOSPHORIC ACID

BY H. B. CLARKE, D. C. CUSWORTH* AND S. P. DATTA

Department of Biochemistry, University College, London

(Received 26 March 1954)

A considerable body of evidence exists indicating that the magnesium salts of many divalent acids such as sulphate (Jones & Monk, 1952), malonate (Evans & Monk, 1952), orthophosphate (Greenwald, Redish & Kibrick, 1940), glycerol 1-phosphate (Tabor & Hastings, 1943) and glucose 1-phosphate (Trevelyan, Mann & Harrison, 1952) are incompletely dissociated in aqueous solution. Only in the cases of magnesium sulphate and magnesium malonate have the thermodynamic dissociations been measured at several temperatures and the thermodynamic quantities associated with the dissociations calculated. As magnesium is known to activate a number of enzyme systems concerned with phosphate metabolism, it is of interest to obtain detailed information about the dissociations of the magnesium salts of those phosphoric acids for which the thermodynamic acid dissociation constants are known.

THEORY

Jones & Monk (1952) have pointed out that the addition of magnesium or other salts of incompletely dissociated acids to cells without liquid junction of

the type Pt, H₂ | HCl | AgCl, Ag will yield information concerning the dissociation of these salts. Accordingly phosphate or phosphate ester buffers containing MgCl₂ were examined potentiometrically in cells without liquid junction of the type

where M is an alkali metal.

The e.m.f. of such a cell is given by

$$E = E_0 - \frac{2 \cdot 3026 RT}{F} \log a_{\text{H}^+} \cdot a_{\text{Cl}^-}. \quad (1)$$

From this equation in terms of molalities and the approximation for the activity coefficients proposed by Güntelberg (1926), the use of which has been discussed previously (Ashby, Crook & Datta, 1954a) we have:

$$\log m_{\text{H}^+} = - \frac{(E - E_0) F}{2 \cdot 3026 RT} - \log m_{\text{Cl}^-} + \frac{2A I^{\frac{1}{2}}}{1 + I^{\frac{1}{2}}}. \quad (2)$$

This equation, together with equation (3), the expression for the dissociation constant K_a of the acid HA⁻:

$$-\log K_a = -\log \frac{m_{\text{H}^+} \cdot m_{\text{A}^{2-}}}{m_{\text{HA}^-}} + \frac{4A I^{\frac{1}{2}}}{1 + I^{\frac{1}{2}}} \quad (3)$$

* Present address, Medical Unit, University College Hospital Medical School.

gives

$$\log m_{A^{1-}} = \log K_s + \log m_{HA^-} + \frac{(E - E_0) F}{2 \cdot 3026 RT} + \log m_{Cl^-} + \frac{2AI^{\frac{1}{2}}}{1 + I^{\frac{1}{2}}}, \quad (4)$$

where $m_{HA^-} = m_a - m_{H^+}$, $m_{M^+} = m_a + 2m_b$, $m_{Cl^-} = 2m_s$, and since undissociated MgA is formed

$$m_{Mg^{1+}} = m_s - m_b + m_{H^+} + m_{A^{1-}}$$

$$\text{hence } I = m_a - m_b + 3m_s - 2m_{H^+} + 4m_{A^{1-}}. \quad (5)$$

It is apparent that equation (4) can be solved for $m_{A^{1-}}$ only by using an approximate value for I ; the value of $m_{A^{1-}}$ thus obtained is used in equation (5), and the new value of I is used in equation (4) to obtain a better value of $m_{A^{1-}}$. The iteration is continued until no further change occurs in either $m_{A^{1-}}$ or I , and these final values are then used to obtain the dissociation constant of the salt

$$K_s = \frac{a_{Mg^{1+}} \cdot a_{A^{1-}}}{a_{MgA}}$$

Fig. 1. Titration of dipotassium glucose 1-phosphate with HCl in the presence of NaCl and $MgCl_2$ of equal ionic strength. ●, 25 ml. 0.1 M dipotassium glucose 1-phosphate and 25 ml. 0.3 M-NaCl; ×, 25 ml. 0.1 M dipotassium glucose 1-phosphate and 25 ml. 0.1 M- $MgCl_2$.

by the equation

$$-\log K_s + \beta I = -\log m_{Mg^{1+}} - \log m_{A^{1-}} + \log m_{MgA} + \frac{8AI^{\frac{1}{2}}}{1 + I^{\frac{1}{2}}} = y, \quad (6)$$

where

$$m_{MgA} = m_b + m_{H^+} - m_{A^{1-}},$$

and

$$m_{Mg^{1+}} = m_s - m_{MgA}.$$

The right-hand side of equation (6), designated by y , is plotted against I and gives a straight line of slope β ; the intercept at $I=0$, giving $-\log K_s$, is obtained by the method of least squares.

The values of $-\log K_s$ at different temperatures vary with the absolute temperature according to the equation of Harned & Robinson (1940),

$$-\log K_s = A/T - D + CT. \quad (7)$$

The thermodynamic quantities associated with the dissociation can be calculated from the constants of equation (7).

It will have been noticed in the above treatment that the possibility of the formation of undissociated complexes of the type $MgHA^+$ or $Mg(HA)_2$ is

Fig. 2. Titration of disodium glycerol 2-phosphate with HCl in the presence of NaCl and $MgCl_2$ of equal ionic strength. ●, 25 ml. 0.1 M disodium glycerol 2-phosphate and 25 ml. 0.3 M-NaCl; ×, 25 ml. 0.1 M disodium glycerol 2-phosphate and 25 ml. 0.1 M- $MgCl_2$.

Table 1. *Values of $-\log K_{2a}$ used*

Temp. (°)	Phosphoric acid	Glucose 1-phosphoric acid	Glycerol 2-phosphoric acid
10	7.254	6.5005	6.6496
20	7.213	6.4997	6.6464
30	7.190	6.5103	6.6565
40	7.182	6.5311	6.6786
50	7.184	6.5613	6.7115

Values for phosphoric acid from Bates & Acree (1943), for glucose 1-phosphoric acid from Ashby, Clarke, Crook & Datta (in preparation) and for glycerol 2-phosphoric acid from Ashby, Crook & Datta (1954*b*). Values of $-\log K_{2a}$ from our own data were used to four decimal places, though the fourth figure is not significant.

ignored. Should such complexes be formed, the titration curves of the acids in the presence of MgCl_2 would be displaced with respect to those in the presence of NaCl of equal ionic strength in the region of the $\text{p}K_1$ as well as in the region of the $\text{p}K_2$. Data exists showing that no such displacement occurs in the region of the $\text{p}K_1$ of phosphoric acid (Greenwald *et al.* 1940), and these authors conclude that no significant amount of the undissociated complexes $\text{MgH}_2\text{PO}_4^+$ or $\text{Mg}(\text{H}_2\text{PO}_4)_2$ occur. Figs. 1 and 2 show titration curves for glucose 1-phosphate and glycerol 2-phosphate in the presence of MgCl_2 and NaCl ; from these curves it may be concluded that in the case of these acids also no significant amounts of undissociated complexes of magnesium and the singly charged acid species occur. It is of interest to note that the association of Mg^{2+} and HSO_4^- ions is considered to be negligible (Jones & Monk, 1952) and that the dissociation constant of the $(\text{Mg}\cdot\text{H malonate})^+$ ion pair is approximately 0.4 (Cannan & Kibrick, 1938). Ion associations between Mg^{2+} , Na^+ and Cl^- are known to be negligibly small, and in the solutions studied here m_{OH^-} was so small that the formation of the complex $\text{Mg}(\text{OH})^+$ for which $K = 0.0026$ (Stock & Davies, 1948) could be neglected. Tabor & Hastings (1943) have given evidence from conductance measurements that the complex formed when MgCl_2 and Na_2HPO_4 solutions are mixed is in fact MgHPO_4 .

The values of the acid dissociation constants used in this work are listed in Table 1.

METHODS

The apparatus, general technique, fundamental constants and the preparation and analysis of the HCl , dipotassium glucose 1-phosphate and disodium glycerol 2-phosphate were the same as have been described previously (Ashby, Crook & Datta, 1954*a*, *b*; Ashby, Clarke, Crook & Datta, in preparation). The temperature was measured with a platinum resistance thermometer constructed in our laboratory and calibrated by the National Physical

Laboratory. The resistance at the triple point of water was frequently checked.

Carbonate-free NaOH was prepared from a saturated solution of washed AnalaR NaOH pellets in water which was suitably diluted with CO_2 -free conductivity water in an apparatus which allowed the dilution to be made in an atmosphere of N_2 , free from CO_2 . The NaOH solution was standardized on both the molar and the molal scales by volumetric and weight titrations with accurately diluted constant-boiling HCl which had itself been checked gravimetrically.

HCl , approx. 1M, was prepared from redistilled approx. 5M- HCl (AnalaR) and standardized by titration with the standard NaOH .

AnalaR KH_2PO_4 was recrystallized 3 times from conductivity water and dried *in vacuo* at 100° .

MgCl_2 solutions were prepared from AnalaR $\text{MgCl}_2 \cdot 5\text{H}_2\text{O}$ and standardized on the molal scale by gravimetric chloride estimations which agreed to within 0.1%. MgCl_2 solutions of known molar concentrations were prepared by weighing out the required amount of solution of known molality and making up to volume.

The titration curves (Figs. 1 and 2) were obtained with a glass electrode (Doran Instrument Co. Alkaglass electrode) and an $\text{Ag}\cdot\text{AgCl}$ electrode in saturated KCl as a reference using a saturated KCl bridge. The electrode assembly was standardized with 0.05M potassium hydrogen phthalate (British Standards Institution (1950) 1647). All solutions for the titration curves were prepared in grade A volumetric apparatus and the HCl was added from a 10 ml. burette. The e.m.f. of the glass electrode assembly was measured with a Tinsley Vernier potentiometer (H. Tinsley and Co., London, S.E. 26) using an electrometer valve impedance converter.

RESULTS

The measured e.m.f.'s, together with the values of the molalities of the various ionic species and the values of y (equation 6), are given in Tables 2-4. The extrapolated values of $-\log K_s$ at infinite dilution, together with the values calculated from equation (7), are given in Table 5. The values of the constants A , C and D of equation (7), together with the minimum $\text{p}K_s$ values and the temperatures at which they occur, are given in Table 6. Where two sets of dilutions were done for one substance, the values of A , C and D are derived from the mean values of the coefficients of the polynomials used for fitting the curves through the points. In both cases there was no significant difference between the two sets of values.

It will be observed that the standard deviations of $-\log K_s$ are considerably larger than those of $-\log K_a$ for the ester phosphates (Ashby, Crook & Datta, 1954*b*; Ashby, Clarke, Crook & Datta, in preparation), although the solutions were prepared and the e.m.f. measurements made with the same high degree of accuracy. The reason for this is that in the measurement of the acid dissociation constants the e.m.f. enters only into the value of $\log m_{\text{H}^+}$ and in the case of the first dissociation into $\log m_{\text{HA}^-}$.

Table 2. *Magnesium phosphate. Molalities (m) and ionic strengths (I) of solutions, e.m.f.'s of cells (E), international volts (corrected to 1 atm. pressure of hydrogen and rounded temperatures) and extrapolation functions (y)*

$$m_a = \text{molality } \text{KH}_2\text{PO}_4, m_b = \text{molality } \text{KNaHPO}_4, m_s = \text{molality } \text{MgCl}_2, m_{\text{H}^+} = \text{antilog} \left[\frac{-(E - E_0)F}{2.3026RT} - \log m_{\text{Cl}^-} + \frac{2AI^{\frac{1}{2}}}{1 + I^{\frac{1}{2}}} \right],$$

$$m_{\text{A}^{2-}} = \text{antilog} \left[\log K_a + \log m_{\text{HA}^-} - \log m_{\text{H}^+} + \frac{4AI^{\frac{1}{2}}}{1 + I^{\frac{1}{2}}} \right]. \quad I = m_a - m_b + 3m_s - 2m_{\text{H}^+} + 4m_{\text{A}^{2-}},$$

$$y = -\log m_{\text{Mg}^{2+}} - \log m_{\text{A}^{2-}} + \log m_{\text{MgA}} + \frac{8AI^{\frac{1}{2}}}{1 + I^{\frac{1}{2}}}. \quad \text{In these solutions } m_{\text{Cl}^-} = 2m_s.$$

Set 1

		0.57459	1.0478	1.4015	1.7630	2.3526	2.6700	3.1520	3.7765
	$10^3 m_a$	0.46990	0.85688	1.1462	1.4418	1.9239	2.1835	2.5777	3.0884
	$10^3 m_b$	0.34802	0.63463	0.84886	1.0678	1.4249	1.6171	1.9091	2.2873
	$10^2 m_s$	2	3	3	4	5	5	5	6
	$10^2 m_{\text{H}^+}$								
10°	E	0.73879	0.71896	0.70936	0.70160	0.69158	0.68721	0.68161	0.67527
	$10^3 m_{\text{A}^{2-}}$	0.30176	0.47574	0.59659	0.70983	0.87682	0.96433	1.0999	1.2588
	$10^2 I$	1.1752	2.1132	2.8107	3.5194	4.6682	5.2857	6.2246	7.4342
	y	2.6146	2.6321	2.6346	2.6427	2.6617	2.6691	2.6749	2.6896
20°	E	0.74747	0.72666	0.71654	0.70846	0.69804	0.69336	0.68766	0.68102
	$10^3 m_{\text{A}^{2-}}$	0.28966	0.45212	0.56325	0.66928	0.82586	0.90296	1.0344	1.1810
	$10^2 I$	1.1704	2.1038	2.7974	3.5032	4.6478	5.2612	6.1984	7.4031
	y	2.6701	2.6895	2.6956	2.7031	2.7209	2.7322	2.7343	2.7503
30°	E	0.75589	0.73406	0.72341	0.71498	0.70414	0.69947	0.69336	0.68644
	$10^3 m_{\text{A}^{2-}}$	0.27340	0.42215	0.52254	0.61949	0.76311	0.84019	0.95504	1.0863
	$10^2 I$	1.1638	2.0918	2.7811	3.4833	4.6227	5.2360	6.1666	7.3652
	y	2.7410	2.7609	2.7690	2.7761	2.7930	2.7985	2.8059	2.8238
40°	E	0.76365	0.74099	0.72985	0.72111	0.70986	0.70499	0.69859	0.69145
	$10^3 m_{\text{A}^{2-}}$	0.25165	0.38747	0.47748	0.56578	0.69625	0.76558	0.86787	0.99041
	$10^2 I$	1.1551	2.0779	2.7630	3.4618	4.5960	5.2062	6.1318	7.3268
	y	2.8321	2.8427	2.8509	2.8565	2.8720	2.8779	2.8867	2.9018
50°	E	0.77098	0.74761	0.73593	0.72684	0.71510	0.71015	0.70331	0.69592
	$10^3 m_{\text{A}^{2-}}$	0.22862	0.35270	0.43210	0.51105	0.62652	0.69109	0.77725	0.88697
	$10^2 I$	1.1459	2.06040	2.7449	3.4399	4.5681	5.1764	6.0955	7.2855
	y	2.9275	2.9267	2.9361	2.9414	2.9578	2.9612	2.9747	2.9894

Set 2

		1.2137	1.6274	2.0995	2.4093	2.9098	3.4685
	$10^3 m_a$	1.0918	1.4640	1.8887	2.1674	2.6175	3.1202
	$10^3 m_b$	0.89066	1.1943	1.5407	1.7681	2.1353	2.5453
	$10^2 m_s$	3	4	4	5	5	6
	$10^2 m_{\text{H}^+}$						
10°	E	0.70953	0.69956	0.69078	0.68626	0.67964	0.67342
	$10^3 m_{\text{A}^{2-}}$	0.54838	0.68407	0.82759	0.92716	1.0666	1.2143
	$10^2 I$	2.9034	3.8727	4.9742	5.6991	6.8616	8.1564
	y	2.6516	2.6619	2.6750	2.6752	2.6897	2.7054
20°	E	0.71667	0.70629	0.69710	0.69243	0.68557	0.67909
	$10^3 m_{\text{A}^{2-}}$	0.51685	0.64372	0.77626	0.87030	1.0016	1.1391
	$10^2 I$	2.8908	3.8566	4.9537	5.6764	6.8356	8.1263
	y	2.7124	2.7219	2.7362	2.7357	2.7493	2.7640
30°	E	0.72348	0.71268	0.70305	0.69826	0.69113	0.68442
	$10^3 m_{\text{A}^{2-}}$	0.47844	0.59473	0.71422	0.80234	0.92261	1.0495
	$10^2 I$	2.8755	3.8370	4.9288	5.6492	6.8040	8.0905
	y	2.7855	2.7941	2.8098	2.8078	2.8213	2.8367
40°	E	0.73000	0.71875	0.70852	0.70361	0.69629	0.68935
	$10^3 m_{\text{A}^{2-}}$	0.43850	0.54374	0.64667	0.72782	0.83891	0.95470
	$10^2 I$	2.8595	3.8166	4.9018	5.6194	6.7705	8.0525
	y	2.8632	2.8717	2.8924	2.8892	2.9005	2.9154
50°	E	0.73599	0.72430	0.71353	0.70851	0.70098	0.69375
	$10^3 m_{\text{A}^{2-}}$	0.39553	0.48948	0.57818	0.65212	0.75292	0.85535
	$10^2 I$	2.8423	3.7948	4.8744	5.5891	6.7362	8.0128
	y	2.9490	2.9571	2.9806	2.9761	2.9863	3.0022

Table 4. *Magnesium glycerol 2-phosphate. Molalities (m) and ionic strengths (I) of solutions, e.m.f.'s of cells (E), international volts (corrected to 1 atm. pressure of hydrogen and rounded temperatures) and extrapolation functions (y)*

m_a = molality NaH glycerol 2-phosphate, m_b = molality Na_2 glycerol 2-phosphate, m_s = molality MgCl_2 ,
 m_{H^+} = antilog $\left[\frac{-(E - E_0)F}{2 \cdot 3026 RT} - \log m_{\text{Cl}^-} + \frac{2A\sqrt{I}}{1 + \sqrt{I}} \right]$, $m_{\text{A}^{1-}}$ = antilog $\left[\log K_a + \log m_{\text{HA}^-} - \log m_{\text{H}^+} + \frac{4A\sqrt{I}}{1 + \sqrt{I}} \right]$,
 $I = m_a - m_b + 3m_s - 2m_{\text{H}^+} + 4m_{\text{A}^{1-}}$, $y = -\log m_{\text{Mg}^{2+}} - \log m_{\text{A}^{1-}} + \log m_{\text{MgA}}$ + $\frac{8A\sqrt{I}}{1 + \sqrt{I}}$. In these solutions $m_{\text{Cl}^-} = 2m_s + m_a$.

Set 1					Set 2			
$10^3 m_a$	1.0899	1.9658	3.2172	4.2023	1.2938	2.4568	3.3851	4.5928
$10^3 m_b$	3.1331	5.6508	9.2482	12.0799	2.9396	5.5824	7.6917	10.4361
$10^2 m_s$	0.48918	0.88227	1.4439	1.8861	0.49039	0.93123	1.2831	1.7409
$10^7 m_{\text{H}^+}$	2	3	3.5	4	2.5	3.5	4.5	5
10°								
E	0.72678	0.70839	0.69229	0.68426	0.72050	0.70050	0.68990	0.68039
$10^3 m_{\text{A}^{1-}}$	2.3786	3.9470	5.8752	7.6355	2.2280	3.8702	5.0062	6.4864
$10^2 I$	2.3236	4.0536	6.4004	8.3440	2.3271	4.2748	5.7595	7.6921
y	2.4096	2.4479	2.5162	2.4959	2.4073	2.4449	2.4913	2.5171
20°								
E	0.73735	0.71823	0.70144	0.69304	0.73081	0.70998	0.69889	0.68894
$10^3 m_{\text{A}^{1-}}$	2.2934	3.7971	5.6303	7.2954	2.1458	3.7118	4.7816	6.1734
$10^2 I$	2.2895	3.9937	6.3025	8.2080	2.2942	4.2114	5.6697	7.5669
y	2.4866	2.5178	2.5837	2.5677	2.4855	2.5182	2.5640	2.5912
30°								
E	0.74783	0.72787	0.71044	0.70165	0.74098	0.71929	0.70783	0.69753
$10^3 m_{\text{A}^{1-}}$	2.1987	3.6173	5.3553	6.9148	2.0501	3.5301	4.5527	5.8813
$10^2 I$	2.2517	3.9217	6.1925	8.0558	2.2559	4.1388	5.5781	7.4500
y	2.5675	2.5977	2.6576	2.6460	2.5708	2.5986	2.6375	2.6616
40°								
E	0.75813	0.73732	0.71919	0.71000	0.75100	0.72839	0.71648	0.70569
$10^3 m_{\text{A}^{1-}}$	2.0948	3.4250	5.0527	6.5013	1.9492	3.3350	4.2935	5.5182
$10^2 I$	2.2101	3.8448	6.0715	7.8904	2.2156	4.0608	5.4744	7.3048
y	2.6521	2.6809	2.7376	2.7301	2.6568	2.6828	2.7190	2.7455
50°								
E	0.76828	0.74658	0.72773	0.71821	0.76068	0.73726	0.72478	0.71395
$10^3 m_{\text{A}^{1-}}$	1.9727	3.2021	4.7058	6.0508	1.8198	3.1072	3.9749	5.1738
$10^2 I$	2.1613	3.7557	5.9327	7.7103	2.1638	3.9696	5.3470	7.1670
y	2.7464	2.7741	2.8277	2.8208	2.7603	2.7777	2.8152	2.8270

(equations 2 and 3), while in the present case the e.m.f. measurement is used to estimate $m_{\text{A}^{1-}}$, which is then used to estimate $m_{\text{Mg}^{2+}}$ and m_{MgA} , and it is obvious from equation (6) that any error in the evaluation of $m_{\text{A}^{1-}}$ will be magnified in $-\log K_a$. Further, $m_{\text{A}^{1-}}$ enters as an important term in the ionic strength (equation 5). Thus it is to be expected that for a given accuracy in the experimental part of this work the errors of the values of $-\log K_a$ will be an order of magnitude greater than errors in $-\log K_a$.

In Table 8 are collected values for the dissociation constants of the magnesium salts studied in this work and some others which may be of interest to biochemists. The thermodynamic quantities associated with the dissociations of these magnesium salts are given in Table 7. The methods of calculating the standard deviations of $-\log K_a$ and of the thermodynamic quantities have been described previously (Please, 1954).

DISCUSSION

The results given in Table 8 show that the magnesium salts of glucose 1-phosphoric acid and glycerol 2-phosphoric acid are very similar in their dissociation constants; MgHPO_4 has a somewhat lower dissociation constant. Magnesium sulphate and magnesium malonate dissociate to much the same extent as the three magnesium phosphates. The values of $-\log K_a$ of both MgHPO_4 and magnesium glucose 1-phosphate calculated from the results of Trevelyan *et al.* (1952) are larger than those found in this work. This may be due in part to their use of magnesium sulphate as a source of Mg^{2+} , the erroneous assumption being made that this salt is fully dissociated. From the known value of the dissociation constant of magnesium sulphate, it may be calculated that in the experiments of Trevelyan *et al.* (1952), where the concentration of

Table 5. Observed and calculated values of $-\log K$ at rounded temperatures together with values of ' β ' the slope of the extrapolation

Calculated values are from $-\log K_s = A/T - D + CT$, where T is the absolute temperature and A , C and D are constants fitted to the observed values by the method of least squares. The standard deviation, σ , of the calculated values is derived as described by Please (1954). Values of $-\log K_s$ (obs.) are given to four decimal places, as these figures were used to calculate the values of the constants A , C and D , and $-\log K_s$ (calc.).

Temp. (°)	Magnesium phosphate				Magnesium glucose 1-phosphate				Magnesium glycerol 2-phosphate			
	Set 1		Set 2		$-\log K_s$ (obs.)	$-\log K_s$ (calc.)	β	$-\log K_s$ (obs.)	$-\log K_s$ (calc.)	β	$-\log K_s$ (obs.)	$-\log K_s$ (calc.)
	$-\log K_s$ (obs.)	β	$-\log K_s$ (obs.)	β								
10	2.6033	1.18	2.6229	0.99	2.3839	2.612	1.31	2.3823	2.386	1.61	2.3587	2.371
20	2.6601	1.25	2.6851	0.96	2.4507	2.674	1.31	2.4582	2.448	1.55	2.4368	2.447
30	2.7317	1.26	2.7583	0.95	2.5262	2.745	1.33	2.5400	2.520	1.51	2.5289	2.532
37	—	—	—	—	—	2.801	—	—	2.575	—	—	2.595
40	2.8192	1.12	2.8367	0.97	2.5892	2.826	1.56	2.6241	2.600	1.52	2.6152	2.624
50	2.9081	1.08	2.9225	0.99	2.6941	2.916	1.42	2.7198	2.690	1.48	2.7283	2.723
$\sigma = \pm 0.003$												
$\sigma = \pm 0.007$												
$\sigma = \pm 0.015$												

Table 6. Constants of the equation

$$-\log K_s = A/T - D + CT,$$

where T is the absolute temperature, together with the minimum $-\log K_s$ values and the temperature at which these occur

Constant	Magnesium phosphate	Magnesium glucose 1-phosphate	Magnesium glycerol 2-phosphate
A	1305	1288	1058
D	8.185	8.298	7.131
C	0.02186	0.02166	0.02036
$-\log K_s$ (min.)	2.50	2.27	2.15
T (min.)	245° K	240° K	230° K

magnesium sulphate was 0.03M, between one-quarter and one-third of the magnesium sulphate would not have been dissociated.

The value for $-\log K_s$ of MgHPO_4 of Tabor & Hastings (1943) by a conductometric method is in good agreement with the value found by the potentiometric method, and the value of $-\log K_s$ these authors give for magnesium glycerol 1-phosphate is close to the potentiometric value for magnesium glycerol 2-phosphate.

The values of $-\log K_s$ values for MgATP^{2-} , MgADP^- and magnesium phosphopyruvate of Burton & Krebs (1953) are higher than those for other magnesium phosphates. It is to be noted that these values are extremely sensitive to ionic strength and the values of $-\log K_s$ at infinite dilution calculated from the Güntelberg equation for the activity coefficients emphasize both the lower degree of dissociation and the sensitivity of $-\log K_s$ values to ionic strength.

A comparison of the thermodynamic data for the dissociation of magnesium sulphate, magnesium malonate and the three magnesium phosphates given in Table 7, shows that the values of ΔG^0 , $-\Delta H^0$ and $-\Delta S^0$ are very similar except for the values of $-\Delta H^0$ and $-\Delta S^0$ for magnesium sulphate which are considerably larger than those for the other compounds. ΔG^0 at 20° for the dissociation of MgATP^{2-} , MgADP^- and magnesium phosphopyruvate are 7.27, 5.96 and 5.19 kcal./mole respectively, which are markedly higher than those for the other magnesium salts.

The importance of complex formation between magnesium and phosphates in studies of equilibria in biological systems has been discussed by Trevelyan *et al.* (1952) and Burton & Krebs (1953). It is also of interest to note that magnesium ions activate phosphatases in the pH region where divalent ester phosphate ions predominate (Roche, 1950), and it is apparently only with such divalent ester phosphate ions that magnesium forms undissociated complexes.

Table 7. *Thermodynamic quantities associated with the dissociations of the magnesium salts of phosphoric acid, glucose 1-phosphoric acid, glycerol 2-phosphoric acid, sulphuric acid and malonic acid*

σ = the standard error. The values for magnesium sulphate from Jones & Monk (1952), and for magnesium malonate from Evans & Monk (1952).

	Temp. (°)	ΔG°		ΔH°		ΔS°		ΔC_P°	
		Cal.	Joule	Cal.	Joule	Cal. deg. ⁻¹	Joule deg. ⁻¹	Cal. deg. ⁻¹	Joule deg. ⁻¹
Magnesium phosphate	25	3695 $\sigma 3.1$	15 460 13	-2917 42	-12 200 176	-22.2 0.15	-92.9 0.6	-60 7	-251 29
	37	3974 $\sigma 3.1$	16 620 13	-3640 43	-15 200 180	-24.6 0.17	-102.9 0.7	-62 7.3	-259 31
Magnesium glucose 1-phosphate	25	3382 $\sigma 7.2$	14 150 30	-2913 96	-12 190 402	-21.1 1.0	-88.3 4.2	-59 16	-247 67
	37	3654 $\sigma 7.3$	15 290 31	-3640 98	-15 200 410	-23.5 1.0	-98 4.2	-61.5 17	-257 71
Magnesium glycerol 2-phosphate	25	3392 $\sigma 14$	14 190 59	-3437 197	-14 380 824	-22.9 0.7	-95.8 2.9	-56 33	-234 138
	37	3680 $\sigma 15$	15 390 63	-4110 200	-17 190 840	-25.1 0.7	-105 2.9	-58 34	-243 140
Magnesium sulphate	25	3200	13 400	-5700	-24 000	-31.0	-130	—	—
Magnesium malonate	25	3900	16 300	-3200	-13 400	-23.9	-100	—	—

Table 8. *The dissociation constants of a number of magnesium salts given in the literature together with their calculated thermodynamic dissociation constants and the method by which they were calculated*

Salt	$-\log K_s$			Quoted value of I	Temp. (°)	Remarks	Author
	Quoted value	Calculated value $I=0$	This paper $I=0$				
Magnesium phosphate	1.52	2.99	2.745	0.2	30	Calc. by Güntelberg eqn. $\beta=1$	1
	2.87	—	2.81	0	38	By conductivity	2
	2.50	—	2.67 (20°)	0	Room temp.	From author's equation	3
Magnesium glucose 1-phosphate	1.29	2.83	2.52	0.2	30	Calc. by Güntelberg eqn. $\beta=1.33$	1
Magnesium glycerol 2-phosphate	—	—	2.60	—	38	—	2
Magnesium glycerol 1-phosphate	1.28	2.60	—	0.16	38	By conductivity. Calc. by Güntelberg eqn. $\beta=1.65$	2
Magnesium ATP ²⁻	2.92	5.42 (20°)	—	0.2	—	Calc. by Güntelberg eqn. $\beta=0$	4
Magnesium ADP ⁻	2.57	4.44 (20°)	—	0.2	—	Calc. by Güntelberg eqn. $\beta=0$	4
Magnesium phospho- pyruvate	2.0	3.87 (20°)	—	0.2	—	Calc. by Güntelberg eqn. $\beta=0$	4
Magnesium malonate	2.936	—	—	0	37	From authors' equation	6
Magnesium sulphate	2.516	—	—	0	37	Graphically from authors' data	5

1, Trevelyan, Mann & Harrison (1952); 2, Tabor & Hastings (1943); 3, Greenwald, Redish & Kibrick (1940); 4, Burton & Krebs (1953); 5, Jones & Monk (1952); 6, Evans & Monk (1952).

SUMMARY

1. The thermodynamic dissociation constants of magnesium phosphate, magnesium glucose 1-phosphate and magnesium glycerol 2-phosphate on the molal scale have been measured at 10° intervals from 10° to 50° using hydrogen and silver-silver chloride electrodes in cells without liquid junction.

2. From these measurements have been calculated the standard free energy, heat content, entropy and heat capacity for the ionizations at 25 and 37°.

3. The magnitude and sources of error are discussed.

4. Sufficient information has been given for the

dissociations of these salts to be considered quantitatively in any kinetic or other study of phosphate, glucose 1-phosphate or glycerol 2-phosphate in the presence of magnesium ions.

The authors wish to thank Dr E. M. Crook for his invaluable advice and encouragement. They also wish to thank the Central Research Fund of the University of London for a grant to purchase the potentiometric equipment used in this work. One of the authors (H. B. C.) thanks the Department of Scientific and Industrial Research for a personal grant.

REFERENCES

- Ashby, J. H., Crook, E. M. & Datta, S. P. (1954*a*). *Biochem. J.* **56**, 190.
 Ashby, J. H., Crook, E. M. & Datta, S. P. (1954*b*). *Biochem. J.* **56**, 198.
 Bates, R. C. & Acree, S. F. (1943). *J. Res. nat. Bur. Stand.* **30**, 129.
 Burton, K. & Krebs, H. A. (1953). *Biochem. J.* **54**, 94.
 Cannan, R. K. & Kibrick, A. C. (1938). *J. Amer. chem. Soc.* **60**, 2314.
 Evans, J. I. & Monk, C. B. (1952). *Trans. Faraday Soc.* **48**, 934.
 Greenwald, I., Redish, J. & Kibrick, A. C. (1940). *J. biol. Chem.* **135**, 65.
 Güntelberg, E. (1926). *Z. phys. Chem.* **123**, 199.
 Harned, H. S. & Robinson, R. A. (1940). *Trans. Faraday Soc.* **36**, 973.
 Jones, H. W. & Monk, C. B. (1952). *Trans. Faraday Soc.* **48**, 929.
 Please, N. W. (1954). *Biochem. J.* **56**, 196.
 Roche, J. (1950). In *The Enzymes*, **1** (1), 473. Ed. J. B. Sumner and K. Myrback. Academic Press Inc.
 Stock, D. I. & Davies, C. W. (1948). *Trans. Faraday Soc.* **44**, 856.
 Tabor, H. & Hastings, A. B. (1943). *J. biol. Chem.* **148**, 627.
 Trevelyan, W. E., Mann, P. F. E. & Harrison, J. S. (1952). *Arch. Biochem. Biophys.* **39**, 440.

The Estimation of Lactic Acid using Ceric Sulphate

By S. R. ELSDEN AND Q. H. GIBSON

*Agricultural Research Council Unit of Microbiology, Department of Microbiology;
 and Department of Physiology, University of Sheffield*

(Received 6 March 1954)

Gordon & Quastel (1939) showed that ceric sulphate oxidizes lactic acid according to the following equation:

and they made this reaction the basis of a method for the estimation of lactic acid. The reaction was carried out at 50° and the acetaldehyde so formed was removed by a stream of nitrogen, trapped in bisulphite and estimated iodometrically in the usual way. The advantages claimed for this procedure were simplicity and the fact that very few compounds gave rise to volatile aldehydes under the experimental conditions recommended by the authors. Glucose in particular did not interfere and a preliminary treatment of glucose-containing test solutions with the copper-lime reagent was not considered necessary.

In its essentials the method devised by Long (1946) was similar to that of Gordon & Quastel (1939). He showed, however, that ceric sulphate oxidizes acetaldehyde to acetic acid; but in spite of this he was able to obtain good yields of acetaldehyde from lactic acid by aerating very rapidly, thus removing the acetaldehyde almost as fast as it was formed. The aeration rate recommended was 500 ml. air/min. as compared with the three to four

bubbles nitrogen/sec. advised by Gordon & Quastel.

Winnick (1942) carried out the reaction in Conway vessels with bisulphite in the central chamber to trap the acetaldehyde and incubated for 2 hr. at 50°. His method was modified by Conway (1950), who carried out the oxidation in stoppered test tubes. After 30 min. incubation at 37°, he transferred 1 ml. samples of the reaction mixture to diffusion vessels where the acetaldehyde was trapped in bisulphite placed in the centre chamber.

In view of the widely divergent conditions recommended by the various authors, it seemed desirable, before using any of the ceric sulphate methods, to examine the various steps in the procedure further. The results of these investigations are given below.

EXPERIMENTAL AND RESULTS

Factors influencing the oxidation of lactic acid by ceric sulphate

Effect of ceric sulphate concentration and temperature on the velocity of the reaction. Since carbon dioxide is one of the products of the reaction, Warburg manometers were used to study the effect of both temperature and concentration of ceric sulphate on the velocity of the reaction. The reaction was carried out in *N* sulphuric acid and 0.2 ml. lithium lactate solution in *N* sulphuric acid was added from the side