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THE VALUE OF ΔG° FOR THE HYDROLYSIS OF ATP*

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SUMMARY

1. The equilibrium constant of the reaction catalysed by glutamine synthetase (L-glutamate:ammonia ligase (ADP), EC. 6.3.1.2),

$$K_{\mathrm{obs}} = \frac{[\mathrm{Gln}]\cdot[\mathrm{ADP}]\cdot[\mathrm{P_i}]}{[\mathrm{Glu}]\cdot[\mathrm{ammonia}]\cdot[\mathrm{ATP}]},$$

was measured at 37 °C between pH 6.6 and 7.6 and $[\mathrm{Mg^{2+}}]$ between 1 and 34 mM.

2. The stability constant of the magnesium–glutamate complex was found to be 7.66 ± 0.26 (S.E.) $\mathrm{M^{-1}}$ at I 0.2 and 37 °C.

3. By combining these results with published data for the equilibrium of the glutaminase reaction, the pK and binding constants of the magnesium complexes with $\mathrm{P_i}$, ADP and ATP, activity coefficients and enthalpy data, the $-\Delta G^\circ$ of the reaction

$$\mathrm{ATPH^{3-} + H_2O \rightleftharpoons ADPH^{2-} + H_2PO_4^{-}},$$

at I 0 and 25 °C was calculated to be 7.53 ± 0.09 (S.E.) kcal/mole (31.5 ± 0.4 kJ/mole).

4. This value is 2.33 kcal/mole less than that calculated by R. C. Phillips, P. George and R. J. Rutman (*J. Biol. Chem.*, 244 (1969) 3330). The difference is caused mainly by (i) the lower value used by us for the equilibrium constant of the glutamine synthetase reaction, (ii) a lower value used for the stability constant of the magnesium–glutamate complex, (iii) allowing for the effect of lowering the temperature from 37 to 25 °C on the RT term.

5. Calculations from data in the literature on the equilibria of the reactions catalysed by hexokinase and glucose-6-phosphatase yield a value similar to that reported above.

6. Data are tabulated and plotted graphically for the value of $-\Delta G^\circ_{\mathrm{obs}}$ at 25 and 37 °C, between pH 6 and 9 and for $\mathrm{Mg^{2+}}$ concentrations up to 50 mM. At pH 7.5, I 0.2 and 25 °C, $\Delta G^\circ_{\mathrm{obs}}$ is -8.48 kcal/mole (-35 kJ/mole), 2.26 kcal/mole less negative than that calculated by Phillips *et al.*

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[*] Dedicated to Professor H. Veldstra on the occasion of his retirement from the chair of biochemistry of the University of Leiden.

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INTRODUCTION

Since the energy required for a large number of biochemical processes is provided by the hydrolysis of ATP to ADP and P_i, the value for the change in the standard free energy, ΔG°, of the reaction

$$ATP + H_2O \rightleftharpoons ADP + P_i \tag{1}$$

is of fundamental importance. The only method available to determine this quantity is from measurements of the equilibrium constant

$$K_{obs} = \frac{[ADP]_e \cdot [P_i]_e}{[ATP]_e},$$

in which the symbols $[ADP]_e$, $[P_i]_e$ and $[ATP]_e$ refer to the total concentrations of the compounds, made up of the various ionic species, including those complexed with magnesium, present at equilibrium. The corresponding change of standard free energy, ΔG°_{obs}, is equal to $-RT\ln K_{obs}$. Since the ionisation constants and the stability constants of the magnesium complexes differ for products and reactants, ΔG°_{obs} is a function of pH and Mg^{2+} concentration.

The value of K_{obs} is so high that equilibrium of Eqn 1 is reached at vanishingly small concentrations of ATP, thus precluding the direct measurement of the equilibrium constant. It is possible, however, to calculate it indirectly by combining data for an ATP-driven synthesis and the hydrolysis of the synthesized product. For example, the sum of Eqns 2 and 3

$$\text{Glutamate} + \text{ammonia} + ATP \rightleftharpoons \text{glutamine} + ADP + P_i \tag{2}$$

$$\text{Glutamine} + H_2O \rightleftharpoons \text{glutamate} + \text{ammonia} \tag{3}$$

is Eqn 1, so that $K_{obs} = K_a \cdot K_b$ where K_a and K_b are the equilibrium constants of the reactions shown in Eqns 2 and 3, respectively.

By combining the data of Levintow and Meister[1] for glutamine synthesis and of Benzinger *et al.*[2] for glutamine hydrolysis, together with new values for ionisation constants and stability constants for magnesium complexes, Alberty[3,4] and Phillips *et al.*[5] have calculated ΔG°_{obs} at various temperatures, Mg^{2+} concentrations, ionic strength and pH values.

The accuracy of these calculations depends upon the reliability of the primary equilibrium data. In view of the fundamental importance of having an accurate value for ΔG°_{obs}, for example for the determination of the phosphate potential of oxidative or photosynthetic phosphorylation[6-11], we thought it desirable to re-examine the value for the equilibrium of the glutamine synthetase reaction used by Alberty[3,4] and Phillips *et al.*[5] in their calculations. Since a value differing considerably from that reported by Levintow and Meister[1] was obtained, a reassessment of the value of ΔG°_{obs} was necessary.

The value for $-\Delta G^\circ_{obs}$, calculated from our data, including also a new determination of the stability constant of the magnesium-glutamate complex near pH 7, is, at pH 7.5, I 0.2 and 25 °C and low Mg^{2+} concentration, 2.3 kcal/mole less negative than that calculated by Phillips *et al.*[5].

RESULTS

The equilibrium constant of the glutamine synthetase reaction

The measurements of the glutamine synthetase reaction were carried out at 37 °C and at various values of pH, ionic strength and Mg^{2+} concentration. In order to reduce the time required to reach equilibrium, all reactants and products were present at zero time and the increase or decrease of the concentration term

$$\frac{[\mathrm{Gln}]\cdot[\mathrm{ADP}]\cdot[\mathrm{P_i}]}{[\mathrm{Glu}]\cdot[\mathrm{ammonia}]\cdot[\mathrm{ATP}]}$$

was followed until a constant value was reached. Illustrative experiments, carried out at pH 6.6, I 0.2 and 50 mM magnesium, are shown in Table I and Fig. 1. In three different experiments, decreasing initial values of the concentration term were used. Within experimental error, the same value was reached after 60 min. The 6 values obtained by combining the data at 60 min and 120 min, together with 6 additional values not shown in Fig. 1, give a mean value for $K_{\mathrm{a\ obs}}$ of 162 ± 5 (S.E.). Fig. 2 shows the results of an experiment, carried out at pH 7.0, I 0.3 and 50 mM magnesium, in which the equilibrium was approached from both sides.

The results of these and other experiments under different conditions are summarized in Table II. The results differ considerably from those reported by Levintow and Meister[1] (1200 at pH 7.0) and Varner and Webster[12] (1700 at pH 7.4).

The stability constant of the magnesium–glutamate complex

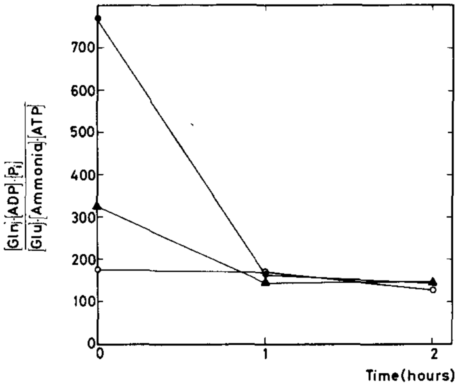
The value of the stability constant of the magnesium-glutamate complex used by Alberty[3,4] and Phillips *et al.*[5] is that reported by Lumb and Martell[13]. However, this value refers to the complex with zero charge, whereas at pH values between

TABLE I

DETERMINATION OF THE EQUILIBRIUM CONSTANT OF THE GLUTAMINE SYNTHETASE REACTION

The reaction mixture contained 100 mM Tris, 50.4 mM $MgCl_2$, 25 mM 2-mercaptoethanol and ATP, ADP, P_i, glutamate, glutamine and ammonia in the amounts given in the table for zero time. The solution was brought to pH 6.6 by addition of KOH. The ionic strength was 0.2. The reactions were carried out in a volume of 3 ml in centrifuge tubes placed in a Dubnoff shaker at 37 °C. The reaction was started by the addition of 1.6 units (µmole/min) glutamine synthetase and stopped by the addition of 0.3 ml 40% $HClO_4$ and 0.1 ml 5% serum albumin. The protein was removed by centrifugation and the supernatant was neutralized with 0.1 M Tris–10% (w/v) KOH, after which the $KClO_4$ was removed by freezing and thawing.

Expt	*Reaction time (min)*	*[Glu] (mM)*	*[Ammonia] (mM)*	*[ATP] (mM)*	*[Gln] (mM)*	*[ADP] (mM)*	*[Pi] (mM)*	$\frac{[Gln]\cdot[ADP]\cdot[P_i]}{[Glu]\cdot[ammonia]\cdot[ATP]}$
1	0	1.00	0.97	0.97	8.8	9.0	9.1	774
	60	1.49	1.73	1.56	8.3	8.4	9.5	165
	120	1.66	1.70	1.62	8.5	8.2	9.5	145
2	0	1.33	1.36	1.36	8.8	9.1	10.0	327
	60	1.55	1.91	1.59	8.3	8.5	10.2	149
	120	1.73	1.90	1.75	8.8	9.5	10.2	147
3	0	1.66	1.68	1.62	8.8	8.8	10.2	175
	60	1.60	1.73	1.61	8.6	9.2	9.7	171
	120	1.75	2.14	1.70	8.7	9.0	10.4	129



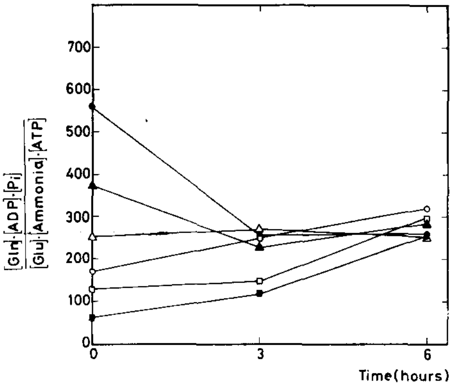


Fig. 1. Determination of the equilibrium constant of the glutamine synthetase reaction at 37 °C, pH 6.6, *I* 0.20 and 50.4 mM $MgCl_2$. Details are given in Table I.

Fig. 2. Determination of the equilibrium constant of the glutamine synthetase reaction at 37 °C, pH 7.0, *I* 0.30 and 50.4 mM $MgCl_2$. The experiment was carried out in the same way as in Table I.

TABLE II

DETERMINATION OF THE EQUILIBRIUM CONSTANT OF THE GLUTAMINE SYNTHETASE REACTION UNDER DIFFERENT CONDITIONS

The mean values reached at equilibrium, under the conditions specified, are given. Temperature, 37 °C.

pH	*I*	*[K]* (*mM*)	*[Mg]* (*mM*)	*[ATP]* (*mM*)	*[ADP]* (*mM*)	*[Pi]* (*mM*)	*[Mg²⁺]* (*mM*)	$K_a{}^*{}_{obs}$
6.6	0.20	45	50.4	1.6	8.8	10.2	34	162
7.0	0.30	98	50.4	1.3	8.8	9.7	34	270
7.1	0.22	93	10.7	1.3	7.6	10.1	2.2	280
7.5	0.21	84	49.5	0.9	9.3	9.4	33	668
7.58	0.17	73	9.8	0.8	9.2	10.1	1.3	831

* $K_{a\ obs}$ = ([Gln]·[ADP]·[Pi])/([Glu]·[ammonia]·[ATP])

6.6 and 7.5, where the measurements of the equilibrium constant of the glutamine synthetase reaction have been carried out, the complex carries one positive charge:

$$\mathrm{Glu}^{2-,1+} + \mathrm{Mg}^{2+} \rightleftharpoons \mathrm{Mg-Glu}^{+} \tag{4}$$

The equilibrium constant of this reaction was determined in two ways, by titration and by an ion-exchange method. In the pH range 3–7, the equilibria given by Eqns 4–6 have to be taken into account.

$$\mathrm{Glu}^{+} \rightleftharpoons \mathrm{Glu}^{-+} + \mathrm{H}^{+} \tag{5}$$

$$\mathrm{Glu}^{-+} \rightleftharpoons \mathrm{Glu}^{2-,1+} + \mathrm{H}^{+} \tag{6}$$

Putting

$$[\mathrm{Mg}] = [\mathrm{Mg}^{2+}] + [\mathrm{Mg\text{-}Glu}^{+}]$$

$$[\mathrm{Glu}] = [\mathrm{Glu}^{+}] + [\mathrm{Glu}^{-+}] + [\mathrm{Glu}^{2-,1+}] + [\mathrm{Mg\text{-}Glu}^{+}]$$

then

$$[\text{Mg–Glu}^+] = [\text{Glu}] - \frac{[\text{Glu}^{2-,1+}]\,[(\text{H}^+)^2 + K_{\text{Glu}^+}\cdot(\text{H}^+) + K_{\text{Glu}^+}\cdot K_{\text{Glu}^{-+}}]}{K_{\text{Glu}^+}\cdot K_{\text{Glu}^{-+}}} \quad (7)$$

where K_{Glu^+} and $K_{\text{Glu}^{-+}}$ are the equilibrium constants of the reactions given in Eqns 5 and 6, respectively, and (H^+) is the activity of H^+.

At pH 7, practically all the glutamate is present as $\text{Glu}^{2-,1+}$. If we titrate a solution at this pH with HCl, the concentration of glutamate that becomes protonated, $[\text{Glu}^{-+}] + [\text{Glu}^+]$, will equal $[\text{HCl}]_{\text{added}}$—$(\text{H}^+)$, *i.e.*

$$[\text{Glu}^{-+}] + [\text{Glu}^+] = [\text{Glu}^{2-,1+}]\left(\frac{(\text{H}^+)}{K_{\text{Glu}^{-+}}} + \frac{(\text{H}^+)^2}{K_{\text{Glu}^+}\cdot K_{\text{Glu}^{-+}}}\right)$$

i.e.

$$[\text{Glu}^{\,2-,1+}] = \frac{K_{\text{Glu}^+}\cdot K_{\text{Glu}^{-+}}\{[\text{HCl}]_{\text{added}} - (\text{H}^+)\}}{K_{\text{Glu}^+}\cdot(\text{H}^+) + (\text{H}^+)^2} \quad (8)$$

If

$$\bar{n} = \frac{[\text{Mg–Glu}^+]}{[\text{Mg}]},$$

$$K_\text{f} = \frac{[\text{Mg–Glu}^+]}{[\text{Mg}^{2+}]\cdot[\text{Glu}^{2-,1+}]} = \frac{\bar{n}}{(1-\bar{n})\cdot[\text{Glu}^{2-,1+}]} \quad (9)$$

The pK values were determined by titration in the absence of magnesium and $\bar{n}$ was calculated from Eqn 10

$$\bar{n} = \frac{[\text{Glu}] - [\text{Glu}^{2-,1+}]\{(\text{H}^+)^2 + K_{\text{Glu}^+}\cdot(\text{H}^+) + K_{\text{Glu}^+}\cdot K_{\text{Glu}^{-+}}\}/K_{\text{Glu}^+}\cdot K_{\text{Glu}^{-+}}}{[\text{Mg}]} \quad (10)$$

and K_f from Eqn 9.

TABLE III

DETERMINATION OF STABILITY CONSTANT OF Mg–GLUTAMATE$^+$ BY TITRATION

50.9 ml of a solution containing 33.9 mM sodium hydrogen glutamate and 57 mM MgCl_2 was titrated with 0.506 M HCl. I 0.2. 25 °C. $K_{\text{Glu}^+} = 5.5\cdot10^{-3}$ M. $K_{\text{Glu}^{-+}} = 7.07\cdot10^{-5}$ M.

HCl added (*ml*)	*pH*	[*Glu*$^{2-,1+}$]* (*mM*)	$\bar{n}$**	K_f*** (M^{-1})
0	6.22			
0.2	5.20	22.1	0.168	9.15
0.4	4.90	22.0	0.134	7.03
0.6	4.70	20.8	0.119	6.52
0.8	4.50	17.3	0.145	9.76
1.0	4.40	17.1	0.113	7.43
1.2	4.29	15.9	0.099	6.96
1.6	4.08	12.9	0.082	6.98
1.8	3.98	11.3	0.075	7.11
2.0	3.88	9.94	0.065	7.03
				Mean 7.55 ± 0.37 (S.E.)

* Calculated by Eqn 8.
** Calculated by Eqn 10.
*** Calculated by Eqn 9.

A typical titration at 25 °C and $I = 0.2$ is given in Table III. The average values for this and two other ionic strengths are given in Table IV. The stability constant at I 0.2 for Mg–Glu$^+$, 7.5 M^{-1}, is much less than the value of 80 M^{-1} given for Mg–Glu° by Lumb and Martell[13] and used by Alberty[3,4] and Phillips *et al.*[5].

The value obtained by the titration method was confirmed by the ion-exchange procedure of Schubert[14]. Using this method, K_f is given by the expression

$$\left(\frac{K_d^\circ}{K_d} - 1\right) \Big/ [\mathrm{Glu}]^n$$

where K°_d and K_d are the distribution coefficients of Mg^{2+} between the ion exchanger and the solution in the absence and presence of glutamate, respectively, and n is the ratio of the number of molecules of metal to ligand in the complex. Assuming $n = 1$, a value of 7.66 ± 0.26 (S.E.) M^{-1} at 37 °C and $I = 0.2$ was obtained (Table V).

TABLE IV

STABILITY CONSTANT OF Mg–GLUTAMATE$^+$ AT DIFFERENT IONIC STRENGTHS, AS DETERMINED BY TITRATION

Method as in Table III.

I	K_{Glu^+} (M)	$K_{\mathrm{Glu}^{-+}}$ (M)	K_f* (M^{-1})
0.08	$4.4 \cdot 10^{-3}$	$4.9 \cdot 10^{-5}$	25.4 ± 1.1
0.2	$5.5 \cdot 10^{-3}$	$7.1 \cdot 10^{-5}$	7.5 ± 0.4
0.27	$5.5 \cdot 10^{-3}$	$7.1 \cdot 10^{-5}$	8.1 ± 0.3

* Mean ± S.E. of mean.

TABLE V

DETERMINATION OF THE STABILITY CONSTANT OF Mg–GLUTAMATE$^+$ BY ION-EXCHANGE METHOD

100 mg Dowex 50-X 8-400 (Sigma) were suspended in 10 ml 15 mM sodium diethylbarbiturate buffer containing 50 μM MgCl$_2$ and varying amounts of sodium hydrogen glutamate and NaCl to bring the ionic strength to 0.21. The pH was brought to 7.4 with HCl and the suspension shaken in a Dubnoff shaker for 3–5 h at 37 °C. Magnesium was then determined with a Perkin–Elmer Atomic Absorption Spectrophotometer Model 305. $K_d{}^\circ$, the distribution coefficient of Mg^{2+} between the ion exchanger and the solution in the absence of glutamate, determined by extrapolation of K_d, the distribution coefficient in the presence of glutamate, to zero glutamate concentration, was equal to 0.23 ml·mg^{-1}.

[Glu] (mM)	[Mg^{2+}] (μM)	K_d ($ml \cdot mg^{-1}$)	K_f* (M^{-1})
39	17.5	0.186	6.08
58	19.4	0.158	7.08
78	20.7	0.142	7.96
117	22.5	0.122	7.58
156	25.0	0.100	8.34
195	26.3	0.0905	7.91
39	18.8	0.171	8.42
78	20.0	0.150	6.85
117	22.5	0.122	7.58
156	25.0	0.100	8.34
			Mean 7.66 ± 0.26 (S.E.)

* $K_f = (K_d{}^\circ / K_d - 1)/[\mathrm{Glu}]$.

Calculation of $\Delta G°_{\mathrm{obs}}$ for the hydrolysis of ATP

Since the ionisation constants and the stability constants of the magnesium complexes differ for products and reactants, $\Delta G°_{\mathrm{obs}}$ will be a function of pH and $[\mathrm{Mg}^{2+}]$. At the high concentrations of K^+ used in our experiments, it is also necessary to consider binding of the reactants and products to this cation. In what follows, we shall restrict ourselves to the pH range 6–9, normally the physiological region. It will be assumed that the pK for the adenine ring is the same in both ATP and ADP. Alberty *et al.*[15] found that this pK equals 4 in both compounds, and Phillips *et al.*[16] found that Mg^{2+} has no effect.

Between pH 6 and 9, the following equilibria for ATP have to be considered:

$$\begin{tikzcd}
 & \mathrm{ATPK}^{3-} \\
 & (w) \arrow[d, leftrightarrow, "K_{\mathrm{K}_1}"] \\
 & \mathrm{K}^+ \\
 & + \\
\mathrm{ATPH}^{3-} \arrow[r, leftrightarrow, "K_1"] & \mathrm{H}^+ + \mathrm{ATP}^{4-} \\
(y) & (z) \\
+ & + \\
\mathrm{Mg}^{2+} \arrow[d, leftrightarrow, "K_4"] & \mathrm{Mg}^{2+} \arrow[d, leftrightarrow, "K_2"] \\
\mathrm{ATPHMg}^{-} \arrow[r, leftrightarrow, "K_3"] & \mathrm{H}^+ + \mathrm{ATPMg}^{2-} \\
(1-w-x-y-z) & (x)
\end{tikzcd}$$

The values for the equilibrium constants at 37 °C, as given in or calculated from the literature, are shown in Table VI, which also includes data for the corresponding equilibrium constants for ADP and $\mathrm{P_i}$.

From the equilibrium equations we may derive (*cf.* ref. 5)

$$y_{\mathrm{ATP}} = \frac{[\mathrm{H}^+]}{K_4[\mathrm{Mg}^{2+}](\mathrm{H}^+) + K_1K_{\mathrm{K}_1}[\mathrm{K}^+] + K_1K_2[\mathrm{Mg}^{2+}] + (\mathrm{H}^+) + K_1}$$

For phosphate the equilibria yield

$$y_{\mathrm{P_i}} = \frac{(\mathrm{H}^+)}{(\mathrm{H}^+) + K_8 + K_9K_{10}[\mathrm{Mg}^{2+}] + K_9K_3[\mathrm{K}^+]}$$

In agreement with Phillips *et al.*[5] we shall use Eqn 11

$$\mathrm{ATPH}^{3-} + \mathrm{H_2O} \rightleftharpoons \mathrm{ADPH}^{2-} + \mathrm{H_2PO_4}^{-} \tag{11}$$

as reference. For this equation

$$K_{\mathrm{ref}} = \frac{[\mathrm{ADPH}^{2-}]\,[\mathrm{H_2PO_4^-}]}{[\mathrm{ATPH}^{3-}]} \tag{12}$$

TABLE VI

IONISATION AND STABILITY CONSTANTS FOR ATP, ADP AND P_i AT 25 °C AND 37 °C AND VARIOUS IONIC STRENGTHS

Reaction	*Constant*	*I 0*		*I 0.17*	*I 0.20*	*I 0.21*	*I 0.22*	*I 0.30*	*Reference*
		25 °C	*37 °C*	*37 °C*	*37 °C*	*37 °C*	*37 °C*	*37 °C*	
$ATPH^{3-} \rightleftharpoons ATP^{4-} + H^+$	K_1	$2.09 \cdot 10^{-8}$	$1.86 \cdot 10^{-8}$	$1.12 \cdot 10^{-7}$	$1.10 \cdot 10^{-7}$	$1.10 \cdot 10^{-7}$	$1.12 \cdot 10^{-7}$	$9.76 \cdot 10^{-8}$	20
$ATP^{4-} + Mg^{2+} \rightleftharpoons ATPMg^{2-}$	K_2	$6.76 \cdot 10^5$	$9.35 \cdot 10^5$	$4.46 \cdot 10^4$	$4.90 \cdot 10^4$	$5.11 \cdot 10^4$	$5.36 \cdot 10^4$	$8.70 \cdot 10^4$	16
$ATPHMg^- \rightleftharpoons ATPMg^{2-} + H^+$	K_3	$4.57 \cdot 10^{-6}$	$4.90 \cdot 10^{-6}$	$6.17 \cdot 10^{-6}$	$5.90 \cdot 10^{-6}$	$5.75 \cdot 10^{-6}$	$5.50 \cdot 10^{-6}$	$4.57 \cdot 10^{-6}$	16
$ATPH^{3-} + Mg^{2+} \rightleftharpoons ATPHMg^-$	K_4	$3.90 \cdot 10^3$	$4.47 \cdot 10^3$	$6.45 \cdot 10^2$	$7.10 \cdot 10^2$	$7.40 \cdot 10^2$	$7.60 \cdot 10^2$	$1.15 \cdot 10^3$	16
$ADPH^{2-} \rightleftharpoons ADP^{3-} + H^+$	K_5	$6.31 \cdot 10^{-8}$	$5.75 \cdot 10^{-8}$	$1.95 \cdot 10^{-7}$	$1.78 \cdot 10^{-7}$	$1.78 \cdot 10^{-7}$	$1.78 \cdot 10^{-7}$	$1.51 \cdot 10^{-7}$	20
$ADP^{3-} + Mg^{2+} \rightleftharpoons ADPMg^-$	K_6	$1.86 \cdot 10^4$	$2.46 \cdot 10^4$	$3.46 \cdot 10^3$	$3.80 \cdot 10^3$	$3.98 \cdot 10^3$	$4.07 \cdot 10^3$	$6.15 \cdot 10^3$	16
$ADPHMg^\circ \rightleftharpoons ADPMg^- + H^+$	K_7	$4.18 \cdot 10^{-6}$	$4.80 \cdot 10^{-6}$	$5.63 \cdot 10^{-6}$	$5.63 \cdot 10^{-6}$	$5.50 \cdot 10^{-6}$	$5.50 \cdot 10^{-6}$	$5.14 \cdot 10^{-6}$	16
$ADPH^{2-} + Mg^{2+} \rightleftharpoons ADPHMg^\circ$	K_8	$2.82 \cdot 10^2$	$3.02 \cdot 10^2$	$1.00 \cdot 10^2$	$1.05 \cdot 10^2$	$1.07 \cdot 10^2$	$1.10 \cdot 10^2$	$1.38 \cdot 10^2$	16
$H_2PO_4^- \rightleftharpoons HPO_4^{2-} + H^+$	K_9	$6.61 \cdot 10^{-8}$	$7.09 \cdot 10^{-8}$	$1.90 \cdot 10^{-7}$	$2.00 \cdot 10^{-7}$	$2.05 \cdot 10^{-7}$	$2.10 \cdot 10^{-7}$	$2.18 \cdot 10^{-7}$	20
$HPO_4^{2-} + Mg^{2+} \rightleftharpoons HPO_4Mg^\circ$	K_{10}	$5.12 \cdot 10^2$	$6.31 \cdot 10^2$	$1.13 \cdot 10^2$	$1.05 \cdot 10^2$	$1.07 \cdot 10^2$	$1.07 \cdot 10^2$	$1.01 \cdot 10^2$	26, 27
$ATP^{4-} + K^+ \rightleftharpoons ATPK^{3-}$	K_{K1}			16	15	15	15	11	28*, 29*
$ADP^{3-} + K^+ \rightleftharpoons ADPK^{2-}$	K_{K2}			7.6	7.6	7.6	7.6	7.6	28*, 29*
$HPO_4^{2-} + K^+ \rightleftharpoons HPO_4K^-$	K_{K3}			4.6	4.6	4.6	4.6	4.6	28*

* Values at 37 °C are calculated using enthalpy data given by Smith and Alberty[28].

From the observed equilibrium constants of the glutamine synthetase and glutaminase reactions we may calculate

$$K_{\text{obs}} = \frac{[\text{ADP}]\cdot[\text{P}_\text{i}]}{[\text{ATP}]} \tag{13}$$

where [ADP] *etc.* indicate total concentrations of all ionic species and complexes of ADP *etc.* Combining Eqns 12 and 13 yields

$$K_{\text{ref}} = \frac{[\text{ADPH}^{2-}]}{[\text{ADP}]}\cdot\frac{[\text{H}_2\text{PO}_4{}^-]}{[\text{P}_\text{i}]}\cdot\frac{[\text{ATP}]}{[\text{ATPH}^{3-}]}\cdot K_{\text{obs}}$$

$$= \frac{y_{\text{ADP}}\cdot y_{\text{P}_\text{i}}}{y_{\text{ATP}}}\cdot K_{\text{obs}} \tag{14}$$

From Eqn 14, $\Delta G°_{\text{ref}}$ may be calculated and, by reversal of the process described above, $\Delta G°_{\text{obs}}$ values at various values of pH and $[\text{Mg}^{2+}]$ may be calculated.

In order to allow for binding of glutamate to Mg^{2+}, under the conditions of measurement of the equilibrium constant of the glutamine synthetase reaction, it is necessary to calculate the concentration of Mg^{2+} unbound to ATP, ADP and P_i. This was calculated as in ref. 3, with the additional correction for the binding of K^+ to ATP, ADP and P_i. The values are listed in the second last column of Table II.

Making use of the stability constant of the magnesium–glutamate complex found at 37 °C (7.66), we can calculate

$$\frac{[\text{Gln}]\cdot[\text{ADP}]\cdot[\text{P}_\text{i}]}{[\text{Glu}^{2+,1-}]\cdot[\text{ammonia}]\cdot[\text{ATP}]} = \frac{[\text{Gln}]\cdot[\text{ADP}]\cdot[\text{P}_\text{i}]}{[\text{Glu}]\cdot[\text{ammonia}]\cdot[\text{ATP}]}\cdot(1 + 7.66[\text{Mg}^{2+}])$$

$$= (1 + 7.66[\text{Mg}^{2+}])K_{\text{a obs}}$$

$$\frac{(\text{Gln})}{(\text{NH}_4\text{Glu})^2}\cdot\frac{[\text{ADP}]\cdot[\text{P}_\text{i}]}{[\text{ATP}]} = \frac{\gamma_{\text{Gln}}}{\gamma^2_{\text{NH}_4\text{Glu}}}(1 + 7.66[\text{Mg}^{2+}])K_{\text{a obs}}$$

where the parentheses indicate activities and γ refers to activity coefficient. The values for the activity coefficients, listed in Table VII, are calculated from the data of Benzinger *et al.*[2].

Even at the higher concentrations of Mg^{2+} used in the experiments summarized in Table VII the amount of magnesium–glutamate complex was a very small fraction of the $[\text{Mg}^{2+}]$, calculated from the concentrations of ATP, ADP and P_i and the stability constants of Mg^{2+} with these compounds.

For K_b (Eqn 3) at 37 °C we have used the value 242 M. This has been calculated from the value of 320 M given by Benzinger *et al.*[2] for 37 °C, with two correction factors. First, a value of $\gamma_{\text{NH}_4\text{Glu}}$ for pH 7 has been used instead of the value appropriate for pH 5 used by Benzinger *et al.*[2]. This resulted in an increase of K_b to 341. Secondly, the factor to convert K_b from 25 to 37 °C was calculated from the heat of

TABLE VII

CALCULATION OF $\Delta G^\circ_{\text{ref}}$.

	Expt No.				
	1	*2*	*3*	*4*	*5*
pH	6.6	7.0	7.1	7.5	7.58
I	0.20	0.30	0.22	0.21	0.17
γ_{Gln}	0.98	0.97	0.98	0.98	0.99
$\gamma_{\text{NH}_4\text{Glu}}$	0.69	0.655	0.675	0.685	0.705
$[\text{Mg}^{2+}]$ (M)	0.0341	0.0341	0.0022	0.0329	0.0013
$K_{\text{a obs}}$	162	270	280	668	831
$K_{\text{obs}} = 242 \cdot (\gamma_{\text{Gln}}/\gamma^2_{\text{NH}_4\text{Glu}}) \cdot (1 + 7.66\ [\text{Mg}^{2+}]) \cdot K_{\text{a obs}}$ (M)					
	$1.04 \cdot 10^5$	$1.99 \cdot 10^5$	$1.49 \cdot 10^5$	$4.27 \cdot 10^5$	$4.08 \cdot 10^5$
y_{ADP}	$10.3 \cdot 10^{-3}$	$3.08 \cdot 10^{-3}$	$39.8 \cdot 10^{-3}$	$1.33 \cdot 10^{-3}$	$21.7 \cdot 10^{-3}$
y_{Pi}	0.208	0.0857	0.185	0.0305	0.0851
y_{ATP}	$1.32 \cdot 10^{-3}$	$0.34 \cdot 10^{-3}$	$5.81 \cdot 10^{-3}$	$0.17 \cdot 10^{-3}$	$3.86 \cdot 10^{-3}$
$K_{\text{ref}} = (y_{\text{ADP}} \cdot y_{\text{Pi}})/(y_{\text{ATP}}) \cdot K_{\text{obs}}$ (M)	$1.68 \cdot 10^5$	$1.54 \cdot 10^5$	$1.89 \cdot 10^5$	$1.02 \cdot 10^5$	$1.95 \cdot 10^5$
$-\Delta G^\circ_{\text{ref, 37 °C}} = 1.419 \log K_{\text{ref}}$ (kcal/mole)					
	7.41	7.36	7.49	7.10	7.51
$-\Delta G^\circ_{\text{ref, 25 °C}} = 1.364 \log K_{\text{ref}} + 0.23$ (kcal/mole)					
	7.36	7.31	7.43	7.06	7.44
$\gamma_{\text{ADPH}^{2-}}/\gamma_{\text{ATPH}^{3-}}$	2.35	1.74	2.24	2.29	2.49
$\gamma_{\text{H}_2\text{PO}_4^-}$	0.66	0.61	0.65	0.655	0.69
$-\Delta G^\circ_{\text{ref, 25 °C}} (I = 0) = -\Delta G^\circ_{\text{ref, 25 °C}} - 1.364 \log (\gamma_{\text{ADPH}^{2-}} \cdot \gamma_{\text{H}_2\text{PO}_4^-})/(\gamma_{\text{ATPH}^{3-}})$					
	7.62	7.34	7.65	7.30	7.76

the reaction reported by Kitzinger and Hems[17]. K_{obs} was calculated from the expression

$$K_{\text{obs}} = \frac{[\text{ADP}] \cdot [\text{P}_\text{i}]}{[\text{ATP}]} = \frac{(\text{Gln}) \cdot [\text{ADP}] \cdot [\text{P}_\text{i}]}{(\text{NH}_4\text{Glu})^2 \cdot [\text{ATP}]} \cdot K_\text{b}$$

$$= \frac{\gamma_{\text{Gln}}}{\gamma^2_{\text{NH}_4\text{Glu}}} (1 + 7.66[\text{Mg}^{2+}]) K_{\text{a obs}} \cdot 242$$

From this value of K_{obs}, K_{ref} was calculated by use of Eqn 14, and $\Delta G^\circ_{\text{ref}}$ by the usual relation between ΔG° and K. These values refer to 37 °C, the temperature at which the measurements of the equilibrium of the glutamine synthetase reaction were carried out.

The K_{ref} for 25 °C was calculated by making use of the van 't Hoff isochore and the values for ΔH for the hydrolysis of ATP given by Kitzinger and Benzinger[18] (—4.8 kcal/mole) and Podolsky and Morales[19] (—4.7 kcal/mole), determined at pH 8. The ΔH_{ref} was calculated from this value according to the equation

$$\Delta H_{\text{ref}} = -4.75 + \frac{K_1/(\text{H}^+)}{1 + K_1/(\text{H}^+)} \cdot \Delta H_1 - \frac{K_5/(\text{H}^+)}{1 + K_5/(\text{H}^+)} \cdot \Delta H_5 - \frac{K_9/(H^+)}{1 + K_9/(\text{H}^+)} \cdot \Delta H_9$$

where ΔH_1, ΔH_5 and ΔH_9 (kcal/mole) are the changes of enthalpy corresponding to the ionization of ATPH^{3-}, ADPH^{2-} and H_2PO_4^-, respectively. These values were calculated from the data of Phillips *et al.*[20]. This yields $\Delta H_{\text{ref}} = -5.9$ kcal/mole at I 0.2. In the absence of sufficient data, no correction was made for a possible effect of ionic strength or binding of K^+, under the conditions of the enthalpy measurements[18,19] (*cf.* ref. 5).

$$\log \frac{K_{\text{ref},25\,°\text{C}}}{K_{\text{ref},37\,°\text{C}}} = \frac{-\Delta H}{2.303\cdot R}\cdot\frac{310-298}{310\cdot 298}$$

$$= \frac{5.9\cdot 10^{3}\cdot 12}{2.303\cdot 1.988\cdot 310\cdot 298}$$

$$-\Delta G^{\circ}_{\text{ref},25\,°\text{C}} = 2.303\cdot 1.988\cdot 298\cdot 10^{-3}\log K_{\text{ref},25\,°\text{C}}\ \text{kcal/mole}$$

$$= 2.303\cdot 1.988\cdot 298\cdot 10^{-3}\log K_{\text{ref},37\,°\text{C}} + \frac{5.9\cdot 12}{310}\ \text{kcal/mole}$$

$$= 2.303\cdot 1.988\cdot 298\cdot 10^{-3}\log K_{\text{ref},37\,°\text{C}} + 0.23\ \text{kcal/mole}$$

$$\therefore -\Delta G^{\circ}_{\text{ref},25\,°\text{C}} + \Delta G^{\circ}_{\text{ref},37\,°\text{C}} = -2.303\cdot 1.988\cdot 12\cdot 10^{-3}\log K_{\text{ref},37\,°\text{C}} + 0.23\ \text{kcal/mole}$$

This correction is treated in detail because it differs from that given by Phillips *et al.*[5], who assumed that $-\Delta G°_{\text{ref},\,25\,°\text{C}} + \Delta G°_{\text{ref},\,37\,°\text{C}} = 0.19$ kcal/mole. This is the term that is a measure of the effect of temperature on K_{ref} (Phillips used the value of -4.75 kcal/mole for ΔH_{ref}) which, in accordance with the principle of Le Chatelier, increases on lowering the temperature of an exothermic reaction. This increase of K, which by itself causes the increase of $-\Delta G°$ by 0.19 kcal/mole, is, however, more than counterbalanced by the decrease in RT on lowering the temperature.

The values given in Table VII are applicable for the ionic strengths given. To calculate to zero ionic strength, the values of K_{ref} given have to be multiplied by

$$\frac{\gamma_{\text{ADPH}^{2-}}\cdot\gamma_{\text{H}_2\text{PO}_4^-}}{\gamma_{\text{ATPH}^{3-}}}$$

which may be equated with $\gamma_{\text{ADPH}^{2-}}\cdot\gamma_{\text{H}_2\text{PO}_4^-}/\gamma_{\text{ADP}^{3-}}$, since it may be assumed that $\gamma_{\text{ATPH}^{3-}} = \gamma_{\text{ADP}^{3-}}$. The quotient $\gamma_{\text{ADPH}^{2-}}/\gamma_{\text{ADP}^{3-}}$ may be obtained from the relationship between pK_b and ionic strength determined by Phillips *et al.*[20]. Values for $\gamma_{\text{H}_2\text{PO}_4^-}$ used in Table VII are the means of the values for KH_2PO_4 and NaH_2PO_4 given by Stokes[21] and Scatchard and Breckenridge[22]. The mean value of the five determinations for $-\Delta G°_{\text{ref},\,25\,°\text{C}}$ at zero ionic strength is 7.53 kcal/mole with a standard error of 0.09. This value is 2.33 kcal/mole lower than that calculated by Phillips *et al.*[5]. Our value for $-\Delta G°_{\text{ref},\,37\,°\text{C}}$ is 2.08 kcal/mole lower than that given by Phillips *et al.*[5].

DISCUSSION

The difference in 2.33 kcal/mole for $\Delta G°_{\text{ref},\,25\,°\text{C}}$ between our value and that of Phillips *et al.*[5] is caused by the following factors:

(1) The equilibrium constant for the glutamine synthetase reaction is lower by a factor of 5 than that determined by Levintow and Meister[1] and used by Phillips *et al.*[5]. This accounts for a difference of 1.0 kcal/mole at 37 °C.

(2) The stability constant for the complex formed between Mg^{2+} and $Glu^{2-,1+}$, the species of glutamate present at the pH values used, is considerably smaller than that between Mg^{2+} and Glu^{2-} used by Phillips *et al.*[5]. At the high Mg^{2+} concentration used by Levintow and Meister[1], this accounts for 0.9 kcal/mole.

(3) In converting the data from 37 °C to 25 °C, Phillips *et al.*[5] did not allow for the effect of lowering temperature on the term RT in calculating ΔG° from K. This amounts to about 0.25 kcal/mole.

(4) We introduced into our calculations binding of K^+ to ATP, ADP and P_i, activity coefficients for glutamine, and the activity coefficient for ammonium glutamate appropriate for pH 7, in calculating the equilibrium of the glutaminase reaction. These points are, however, of minor importance.

Since a major contribution to the difference of 2.33 kcal/mole is the disagreement between our measurements and those of Levintow and Meister[1] of the equilibrium constant of the glutamine synthetase reaction, it is desirable to have independent data from which ΔG for the hydrolysis of ATP can be calculated. Data in the literature for the equilibrium of the hexokinase[23] and glucose-6-phosphatase[24] reactions lend themselves to such a calculation, if certain assumptions may be made.

(1) The second ionisation constant of glucose 6-phosphate and the stability constant of the complex with magnesium are the same as the values given for glucose 1-phosphate, *viz.* $3.1 \cdot 10^{-7}$ M (ref. 25), and $3.32 \cdot 10^{2}$ M^{-1} (ref. 26) at zero ionic strength and 30 °C.

(2) The change in the second ionisation constant with ionic strength is the same as that for the second ionisation constant of AMP[20].

(3) The change with ionic strength of the stability constant of the magnesium complex with glucose 6-phosphate is the same as that with $ADPH^{2-}$ (ref. 16).

Robbins and Boyer[23] found a value of

$$K'_{\text{obs}} = \frac{[\text{Glc-6-}P]\,[\text{ADP}]}{(\text{Glc})\,[\text{ATP}]}$$

of 184 at pH 6.04, I 0.30 and 30 °C, with $[Mg^{2+}] = 80$ mM. Using Eqn 15

$$\text{Glc} + \text{ATPH}^{3-} \rightleftharpoons \text{Glc-6-}P^{-} + \text{ADPH}^{2-} \tag{15}$$

as the reference reaction, in the absence of Mg^{2+}

$$K'_{\text{ref}} = K'_{\text{obs}} \cdot \frac{y_{\text{Glc-6-}P} \cdot y_{\text{ADP}}}{y_{\text{ATP}}}$$

This gives K_{ref} of 248 at I 0.3 and 30 °C and in the absence of any thermochemical data it will be assumed that this value also applies to 25 °C.

Atkinson *et al.*[24] found a

$$K''_{\text{obs}} = \frac{(\text{Glc})\,[\text{P}_i]}{[\text{Glc-6-}P]}$$

of 320 M at pH 7.0, I 0.30 and 25 °C in the absence of Mg^{2+}.

TABLE VIII

VALUES OF $-\Delta G°_{obs}$ (kcal/mole) AT 25 °C FOR VARIOUS IONIC STRENGTHS, pH AND $[Mg^{2+}]$ CONCENTRATIONS

In parentheses are given the $-\Delta G°_{obs}$ values in kJ.

pH	*I*	$[Mg^{2+}]$ (*mM*)				
		0	*1.0*	*10.0*	*25.0*	*50.0*
6.00	0.00	7.59 (31.77)				
6.00	0.10	7.25 (30.33)	6.58 (27.53)	6.17 (25 82)	6.18 (25.85)	
6.00	0.15	7.30 (30.54)	6.64 (27.76)	6.23 (26.05)	6.23 (26.05)	6.30 (26.34)
6.00	0.20	7.37 (30.86)	6.69 (27.97)	6.29 (26.32)	6.30 (26.34)	6.38 (26.08)
6.50	0.00	7.71 (32.27)				
6.50	0.10	7.44 (31.12)	6.51 (27.25)	6.31 (26.39)	6.42 (26.87)	
6.50	0.15	7.49 (31.33)	6.57 (27.49)	6.36 (26.60)	6.46 (27.02)	6.62 (27.69)
6.50	0.20	7.56 (31.62)	6.62 (27.70)	6.43 (26.91)	6.54 (27.38)	6.71 (28.09)
7.00	0.00	8.01 (33.51)				
7.00	0.10	7.82 (32.72)	6.69 (28.00)	6.66 (27.88)	6.87 (28.74)	
7.00	0.15	7.87 (32.91)	6.75 (28.25)	6.71 (28.06)	6.90 (28.85)	7.13 (29.82)
7.00	0.20	7.93 (33.17)	6.80 (28.46)	6.79 (28.40)	6.99 (29.25)	7.23 (30.26)
7.50	0.00	8.55 (35.77)				
7.50	0.10	8.38 (35.05)	7.12 (29.80)	7.20 (30.12)	7.46 (31.20)	
7.50	0.15	8.42 (35.23)	7.18 (30.06)	7.24 (30.28)	7.48 (31.28)	7.74 (32.40)
7.50	0.20	8.48 (35.48)	7.23 (30.27)	7.32 (30.04)	7.58 (31.70)	7.85 (32.85)
8.00	0.00	9.24 (38.67)				
8.00	0.10	9.02 (37.74)	7.71 (32.24)	7.83 (32.74)	8.11 (33.91)	
8.00	0.15	9.06 (37.90)	7.77 (32.51)	7.86 (32.90)	8.12 (33.99)	8.40 (35.16)
8.00	0.20	9.12 (38.16)	7.82 (32.71)	7.95 (33.26)	8.22 (34.41)	8.51 (35.62)
8.50	0.00	9.96 (41.67)				
8.50	0.10	9.69 (40.54)	8.35 (34.96)	8.49 (35.52)	8.78 (36.72)	
8.50	0.15	9.73 (40.70)	8.42 (35.22)	8.53 (35.68)	8.80 (36.82)	9.09 (38.03)
8.50	0.20	9.79 (40.96)	8.47 (35.42)	8.61 (36.04)	8.90 (37.22)	9.19 (38.45)
9.00	0.00	10.66 (44.59)				
9.00	0.10	10.37 (43.38)	9.03 (37.76)	9.17 (38.36)	9.46 (39.56)	
9.00	0.15	10.41 (43.54)	9.09 (38.03)	9.20 (38.50)	9.47 (39.63)	9.76 (40.85)
9.00	0.20	10.47 (43.80)	9.14 (38.23)	9.29 (38.87)	9.58 (40.06)	9.87 (41.31)

Using Eqn 16

$$\text{Glc-6-}P^- + H_2O \rightleftharpoons \text{Glc} + H_2PO_4^- \tag{16}$$

as the reference reaction

$$K''_{\text{ref}} = K''_{\text{obs}} \cdot \frac{y_{P_i}}{y_{\text{Glc-6-}P}}$$

This gives a K''_{ref} of 674 M at 25 °C and I 0.3.

$$K_{\text{ref}} = K'_{\text{ref}} \cdot K''_{\text{ref}}$$

$$= 248 \cdot 674\,\text{M}$$

$$= 1.67 \cdot 10^5\,\text{M}$$

corresponding to $-\Delta G^\circ_{\mathrm{ref},\ 25\ ^\circ\mathrm{C}}$ of 7.11 kcal/mole at I 0.3. Converting to I 0, $-\Delta G^\circ_{\mathrm{ref},\ 25\ ^\circ\mathrm{C}}$ equals 7.14 kcal/mole, which is only just outside the range of values shown in Table VII, and far from the value of 9.9 kcal/mole calculated by Phillips *et al.*[5].

Reversal of the calculation carried out to obtain K_{ref} from K_{obs} yields $-\Delta G^\circ_{\mathrm{obs}}$ values referring to various conditions (Tables VIII and IX). In Fig. 3 is plotted the variation of $-\Delta G^\circ_{\mathrm{obs}}$ with pMg at different pH values.

TABLE IX

VALUES OF $-\Delta G^\circ_{\mathrm{obs}}$ (kcal/mole) AT 37 °C FOR VARIOUS IONIC STRENGTHS, pH AND Mg^{2+} CONCENTRATIONS

In parentheses are given the $-\Delta G^\circ_{\mathrm{obs}}$ values in kJ.

pH	*I*	*[Mg^{2+}] (mM)*				
		0	*1.0*	*10.0*	*25.0*	*50.0*
6.00	0.00	7.69 (32.16)				
6.00	0.10	7.25 (30.34)	6.48 (27.10)	6.14 (25.70)	6.21 (25.98)	
6.00	0.15	7.27 (30.41)	6.48 (27.10)	6.14 (25.70)	6.21 (25.98)	6.34 (26.51)
6.00	0.20	7.32 (30.65)	6.48 (27.10)	6.19 (25.88)	6.27 (26.22)	6.42 (26.85)
6.50	0.00	7.81 (32.69)				
6.50	0.10	7.48 (31.30)	6.46 (27.02)	6.36 (26.63)	6.56 (27.44)	
6.50	0.15	7.51 (31.41)	6.47 (27.07)	6.37 (26.65)	6.55 (27.40)	6.78 (28.35)
6.50	0.20	7.57 (31.67)	6.50 (27.18)	6.44 (26.95)	6.64 (27.80)	6.89 (28.82)
7.00	0.00	8.13 (34.00)				
7.00	0.10	7.92 (33.15)	6.71 (28.09)	6.81 (28.51)	7.10 (29.70)	
7.00	0.15	7.95 (33.28)	6.74 (28.22)	6.82 (28.54)	7.09 (29.65)	7.37 (30.85)
7.00	0.20	8.03 (33.59)	6.79 (28.43)	6.92 (28.94)	7.20 (30.13)	7.50 (31.38)
7.50	0.00	8.70 (36.40)				
7.50	0.10	8.53 (35.71)	7.21 (30.18)	7.42 (31.03)	7.75 (32.41)	
7.50	0.15	8.57 (35.85)	7.26 (30.36)	7.43 (31.07)	7.73 (32.34)	8.04 (33.65)
7.50	0.20	8.65 (36.18)	7.32 (30.63)	7.53 (31.52)	7.86 (32.87)	8.18 (34.21)
8.00	0.00	9.44 (39.49)				
8.00	0.10	9.22 (38.56)	7.84 (32.81)	8.09 (33.85)	8.43 (35.29)	
8.00	0.15	9.25 (38.70)	7.89 (33.02)	8.10 (33.88)	8.42 (35.23)	8.74 (36.56)
8.00	0.20	9.33 (39.04)	7.96 (33.32)	8.21 (34.35)	8.55 (35.76)	8.88 (37.14)
8.50	0.00	10.20 (42.66)				
8.50	0.10	9.92 (41.50)	8.53 (35.67)	8.79 (36.76)	9.18 (38.40)	
8.50	0.15	9.95 (41.63)	8.58 (35.88)	8.79 (36.76)	9.14 (38.24)	9.46 (39.57)
8.50	0.20	10.03 (41.98)	8.65 (36.19)	8.91 (37.28)	9.27 (38.79)	9.60 (40.17)
9.00	0.00	10.93 (45.73)				
9.00	0.10	10.63 (44.46)	9.23 (38.60)	9.49 (39.72)	9.85 (41.20)	
9.00	0.15	10.66 (44.59)	9.28 (38.82)	9.50 (39.75)	9.83 (41.14)	10.17 (42.54)
9.00	0.20	10.74 (44.94)	9.35 (39.13)	9.62 (40.23)	9.96 (41.67)	10.31 (43.14)

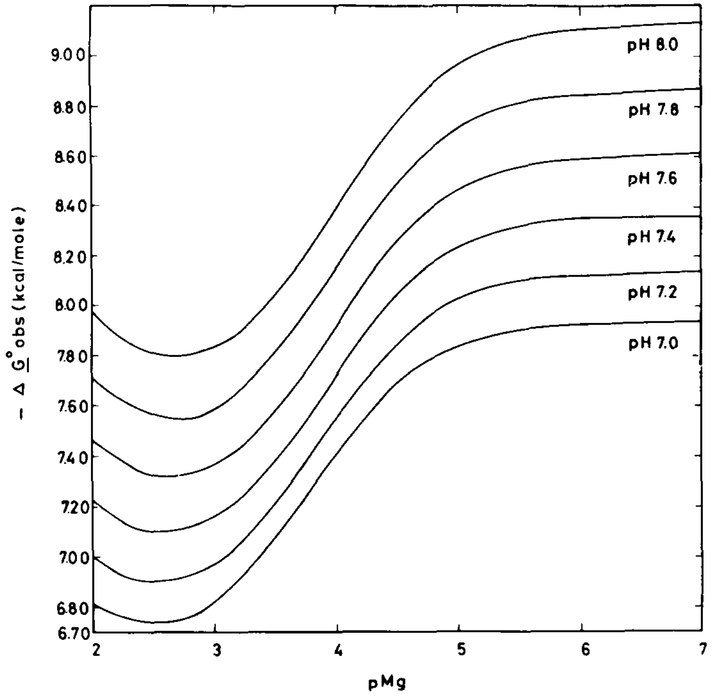


Fig. 3. $\Delta G^\circ_{\mathrm{obs}}$ as function of pMg at I 0.20, 25 °C and various pH values.

METHODS

Glutamine synthetase (L-glutamate:ammonia ligase (ADP), EC 6.3.1.2) was isolated from sheep brain by the method of Ronzio *et al.*[30] up to the step involving chromatography on DEAE-cellulose. This yields a fairly active preparation with an activity equivalent to 5 units (μmole/min) per mg protein, determined by the method of Ronzio *et al.*[30]. It was free from ATPase, adenylate kinase and glutaminase activity.

Phosphate was determined by the method of Sumner[31].

ATP was determined with hexokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) and glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate:NADP$^+$ oxidoreductase, EC 1.1.1.49), ADP with pyruvate kinase (ATP:pyruvate phosphotransferase, EC 2.7.1.40) and lactate dehydrogenase (L-lactate:NAD$^+$ oxidoreductase, EC 1.1.1.27), and glutamate and ammonia with glutamate dehydrogenase (L-glutamate:NAD$^+$ oxidoreductase (deaminating), EC 1.4.1.2). All these analyses were carried out as described by Bergmeyer[32], except that in the ammonia determination Tris buffer was replaced by 0.3 M potassium phosphate buffer (pH 7.5). Glutamine was determined by adding glutaminase (L-glutamine amidohydrolase, EC 3.5.1.2) after completion of the ammonia determination.

Magnesium was determined by titration with EDTA in ammonia–NH_4Cl buffer (pH 10) with Erio Black T as indicator.

The pH was determined with a Radiometer Titrator Type TTT 1c. The electrode was standardized at pH values of 4.0 and 7.0 with buffersolution tablets supplied by Burroughs Welcome and Co., London. The temperature was maintained at 25 ± 0.3 °C.

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