The Equilibrium Constant of the Isocitrate Dehydrogenase Reaction

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1. The equilibrium constant for oxidative decarboxylation of isocitrate by NADP⁺, catalysed by isocitrate dehydrogenase, was measured in solutions of various ionic strengths and at several temperatures. 2. Thermodynamic values for the reaction were obtained by extrapolation to zero ionic strength, and the heat of reaction was estimated. 3. The effect of Mg²⁺ ion concentration on the equilibrium was studied.

The equilibrium constant for the oxidative decarboxylation of isocitrate by NADP⁺ was estimated by Ochoa (1948) as the mean of values obtained at room temperature with various bicarbonate concentrations. The value has been used to calculate standard free-energy data for the reaction (Burton & Wilson, 1953; Burton & Krebs, 1953; Burton, 1957). In the present work, the effects of ionic strength, temperature and Mg²⁺ ion concentration on the equilibrium were studied to determine the equilibrium constant under defined conditions for kinetic experiments and under physiological conditions, and to obtain more precise estimates of standard thermodynamic quantities.

MATERIALS AND METHODS

Enzymes. Isocitrate dehydrogenase [*threo*-D₅-isocitrate-NADP⁺ oxidoreductase (decarboxylating), EC 1.1.1.42] was prepared from ox heart mitochondria by (NH₄)₂SO₄ fractionation, precipitation with nucleic acid, acetone fractionation and a second (NH₄)₂SO₄ fractionation. The specific activity was 25 μ moles of NADP⁺/min./mg. of protein in the assay described by Siebert, Dubuc, Warner & Plaut (1957), which is about half that of the pig heart enzyme isolated by these workers, and twice that of the preparation described by Rose (1960). The enzyme preparation was free from aconitase, glutamate dehydrogenase, NAD-linked isocitrate dehydrogenase and NADPH oxidase activities. Stock solutions of 10 mg./ml. in 0.05M-sodium phosphate buffer, pH 7.4, containing 1 mM-EDTA and 10% (w/v) (NH₄)₂SO₄, were stable at 2°. For use, solutions containing 1–2 mg./ml. were freshly prepared by dilution with the same buffer containing EDTA but no (NH₄)₂SO₄.

Ox liver glutamate dehydrogenase was obtained from C.F. Boehringer und Soehne G.m.b.H. (Mannheim, Germany) as a crystalline suspension in 2.8M-(NH₄)₂SO₄.

Coenzymes. These were obtained from Boehringer Corp. (London) Ltd. (London, W. 5). NADP⁺ was assayed spectrophotometrically with isocitrate dehydrogenase. The

reaction mixture contained, in 3 ml., 80 μ moles of tris-chloride buffer, pH 7.4, 16 μ moles of DL-isocitrate, 4 μ moles of MgSO₄, 1 μ mole of EDTA and about 0.2 μ mole of NADP⁺. With 10 μ g. of enzyme the reaction was complete (99.95%) in 5 min.

NADPH was assayed by complete oxidation with 2-oxoglutarate and glutamate dehydrogenase. The reaction mixture contained 100 μ moles of sodium phosphate buffer, pH 7.4, 3 μ moles of 2-oxoglutarate and about 0.15 μ mole of NADPH in 3 ml. With 10 μ l. of crystalline enzyme suspension in 2.8M-(NH₄)₂SO₄ the reaction was complete in 10 min. The residual extinction at 340 m μ was 1% of the initial value (cf. Dalziel & Dickinson, 1965).

Substrates. DL-Isocitrate was obtained as the trisodium salt from Sigma Chemical Co. (St Louis, Mo., U.S.A.). It was assayed with isocitrate dehydrogenase as described for NADP⁺, except that 0.7 μ mole of NADP⁺ and about 0.2 μ mole of DL-isocitrate were used.

2-Oxoglutaric acid from Koch-Light Laboratories Ltd. (Colnbrook, Bucks.) was recrystallized from acetone-benzene. Solutions were neutralized with NaOH and assayed with glutamate dehydrogenase. The reaction mixture contained, in 3 ml., 100 μ moles of sodium phosphate buffer, pH 7.4, 0.25 μ mole of NADH and about 0.15 μ mole of 2-oxoglutarate. The reaction was complete in 10 min. with 10 μ l. of enzyme.

It was shown by enzymic analysis that the 2-oxoglutarate was free from isocitrate (<0.05%) and that the isocitrate was free from 2-oxoglutarate (<0.5%).

The CO₂ was introduced into the reaction mixtures by equilibration at atmospheric pressure either with the pure gas obtained from a Thermos flask containing solid CO₂ or with CO₂+N₂ (5:95) from a cylinder (British Oxygen Co. Ltd.). The latter was kindly analysed by Dr N. K. Bhattacharyya in the Department of Physiology, Oxford, and the CO₂ content was 5.24%.

Buffer and salts. Salts were A.R. grade from British Drug Houses Ltd. (Poole, Dorset). Reaction mixtures were buffered at pH 6.8–7.2 by the addition of the appropriate concentration of KHCO₃, calculated from the dissolved CO₂ concentration and pK₁' 6.4 for H₂CO₃. The ionic strength was varied by the addition of KCl. It was confirmed that the equilibrium constant, expressed in terms of dissolved CO₂, is independent of pH over this limited range

at constant ionic strength. Greater variation of pH was prohibited by coenzyme instability and most measurements were made at pH 6.9–7.2.

Except in experiments on the effect of Mg^{2+} ion concentration, 0.133M-MgSO₄ was present in all reaction mixtures to supply the essential metal ion for the enzyme.

The ionic strengths of the reaction mixtures were calculated from the concentrations of KHCO₃, KCl, MgSO₄ and 2-oxoglutarate. The contributions of isocitrate, coenzymes and enzyme were neglected.

Equilibrium measurements. Water and stock solutions of KHCO₃ and KCl used to make up the reaction mixtures were first saturated with CO₂. Reaction mixtures, without enzyme and coenzyme, were made up in quartz cells (optical path 1 cm.), sealed with Parafilm, and brought to the required temperature in a water bath. CO₂ was passed first through water at the same temperature and then into the reaction mixture by means of a Teflon tube (1 mm. bore) that passed through a hole in the Parafilm to the bottom of the cell. After 15 min. the tube was withdrawn, coenzyme was added and the cell was sealed again. The extinction at 340 mμ was measured in a Zeiss spectrophotometer (model PMQII), equipped with a thermostatically controlled cell housing. Enzyme (10 μl.) was then added to the 3 ml. reaction mixture, the cell was returned to the water bath, and CO₂ was again passed until the extinction at 340 mμ reached a constant value.

To minimize coenzyme breakdown, relatively large amounts of enzyme (15–50 μg.) were used so that equilibrium was reached in 5 min. at low ionic strengths, and in 15 min. at high ionic strengths, which inhibit the enzyme.

No significant variation of the equilibrium constant with the enzyme concentration was observed. Once equilibrium was reached the extinction in the sealed cell remained constant even without gassing for several minutes if the surface of the solution was not disturbed. To avoid excessive pressure, the CO₂ was passed slowly into the cell, and the delivery tube fitted loosely in an oversize hole in the Parafilm seal. The hydrostatic pressure on the gas bubbles is negligible (<0.1%).

Calculations. The equilibrium was approached from both sides, and the concentration of NADPH at equilibrium was calculated from the change of extinction at 340 mμ. To allow for the small contribution by 2-oxoglutarate to absorption at this wavelength, an extinction coefficient of 6.24×10^6 cm.²/mole was used instead of the value of 6.22×10^6 cm.²/mole for NADPH (Horecker & Kornberg, 1948). The partial pressure of CO₂ was calculated from the barometric pressure and the saturated water-vapour pressure at the temperature of the experiment.

The equilibrium constant for the reaction:

was calculated from the equation:

$$K_p = \frac{[NADPH][2\text{-oxoglutarate}^{2-}]}{[NADP^+][\text{isocitrate}^{3-}]} \times \frac{p}{760} \quad (2)$$

where p is the partial pressure of CO₂ in mm. Hg. This equation can be applied for all ionic strengths, and extrapolation to zero ionic strength at 25° gives the thermodynamic equilibrium constant, K_a , for reaction (1). The variation of K_p with ionic strength will reflect the changing

activity coefficients of dissolved ionic reactants only, and can be interpreted by the Debye-Hückel theory. The standard free-energy change for reaction (1) at 25° was calculated from the reaction isotherm:

$$-\Delta G^0 = 2.303RT \log K_a = 1.364 \log K_a \text{ kcal./mole}$$

and refers to the usual standard states of unit activity of the ideal molar solution for dissolved reactants and 1 atm. pressure for the pure gas. The enthalpy change for reaction (1) was calculated from the effect of temperature on K_p with I 0.104 by using the van't Hoff isochore:

$$d(\log K)/d(1/T) = -\Delta H/2.3R$$

In relation to kinetic and metabolic studies, the equilibrium constant for the reaction:

at a given ionic strength and temperature may be more useful than that for reaction (1), and was calculated from

$$K_c = \frac{[NADPH][2\text{-oxoglutarate}^{2-}][\text{CO}_2]}{[NADP^+][\text{isocitrate}^{3-}]} \quad (4)$$

The dissolved CO₂ concentration was estimated from the partial pressure and the data of Harned & Davis (1943) for the solubility of CO₂ in water and in NaCl solutions over a wide range of temperature. It was assumed that the solubilities of CO₂ in reaction mixtures containing KHCO₃ and KCl as the principle ionic species are the same as in NaCl solutions of the same ionic strength. This is not strictly true, but the error would be negligible over the range of ionic strength used, since the solubility of CO₂ in 0.5 M-NaCl is only 10% less than in water.

Variation of K_c with ionic strength will be partly due to variations of the activity coefficient of CO₂. Extrapolation to zero ionic strength will give the thermodynamic equilibrium constant from which the standard free-energy change for reaction (3) could be calculated. This will refer to a standard state of unit activity of the ideal molar solution of CO₂, as well as of other reactants. However, it is simpler and more accurate to calculate the standard free-energy change for reaction (3) from that for reaction (1) and that for:

The solubility of CO₂ in pure water at 25° is 0.0344 molal at 760 mm. (Harned & Davis, 1943), from which ΔG^0 for reaction (5) is $-1.364 \log 0.0344 = 2.00$ kcal./mole (cf. Burton & Krebs, 1953). Similarly, the enthalpy change for reaction (3) will be the sum of that for reaction (1) and the heat of solution of CO₂, which is $\Delta H^0 = -4.72$ kcal./mole (Harned & Davis, 1943).

RESULTS

The results of equilibrium measurements at 25° with I 0.104 and 0.133M-magnesium sulphate are shown in Table 1. The equilibrium constant is independent of the reactant concentrations, but the average values obtained by approaching the equilibrium from the two sides differ by 10%. The reason for this difference is not known. Although

Table 1. *Isocitrate dehydrogenase equilibrium in potassium hydrogen carbonate-potassium chloride solutions of ionic strength 0.104 and pH 6.9-7.2 at 25°*

Reaction mixtures also contained 0.133 mM-MgSO₄. Equilibrium was approached from both sides: reaction mixtures contained initially either (A) NADP⁺, isocitrate and 2-oxoglutarate or (B) NADPH and 2-oxoglutarate. The concentrations stated are the equilibrium values. Values of K_p are calculated from eqn. (2), and values of K_c from eqn. (4). Concentrations of dissolved CO₂ are calculated from p , the partial pressure of CO₂, and the solubility data of Harned & Davis (1943).

	[NADP ⁺] (μM)	[Isocitrate] (μM)	[NADPH] (μM)	[2-Oxoglutarate] (μM)	p (mm. Hg)	[CO ₂] (mM)	K_p (atm.)	K_c (M)
(A)	56.3	33.7	44.7	1058	737	32.4	24.2	0.81
	56.6	34.0	44.4	1057	737	32.4	23.6	0.79
	52.9	36.2	44.2	1087	740	32.5	24.4	0.81
	54.5	37.8	42.6	1086	740	32.5	21.9	0.72
	33.5	16.8	63.6	275	740	32.5	30.2	1.00
	35.7	19.0	61.4	273	740	32.5	24.1	0.80
	13.1	10.8	36.1	2086	37.3	1.64	26.1	0.88
	13.6	11.3	35.9	2101	37.3	1.64	24.1	0.81
	13.8	11.4	35.4	2080	37.5	1.65	23.1	0.78
	13.3	10.9	35.9	2080	37.5	1.65	25.5	0.85
						Mean	24.7	0.82
(B)	40.2	40.2	45.6	1003	740	32.5	27.6	0.92
	40.1	40.1	45.7	1003	740	32.5	27.8	0.93
	25.5	25.5	60.3	291	740	32.5	26.3	0.88
	24.8	24.8	61.0	292	740	32.5	28.2	0.93
	10.4	10.4	27.5	2002	37.9	1.66	25.4	0.85
	10.3	10.3	27.6	2002	37.9	1.66	26.0	0.86
						Mean	26.9	0.90

Table 2. *Isocitrate dehydrogenase equilibrium in potassium hydrogen carbonate-potassium chloride solutions of various ionic strengths and pH 6.6-7.2 at 25°*

I	pH	[NADP ⁺] (μM)	[Isocitrate] (μM)	[NADPH] (μM)	[2-Oxoglutarate] (μM)	p (mm. Hg)	[CO ₂] (mM)	K_p (atm.)	K_p (av.) (atm.)	K_c (av.) (M)
0.017	7.2	13.2	10.8	36.2	2081	37.4	1.69	26.0	28.3	0.93
		12.5	10.1	36.9	2082	37.4	1.69	30.0		
		9.9	9.9	28.0	2003	37.9	1.71	28.6		
		9.9	9.9	28.0	2003	37.9	1.71	28.6		
0.054	6.6	50.6	37.2	46.5	1070	733	32.8	25.5	26.7	0.90
		49.9	36.7	46.5	1063	733	32.8	26.0		
		34.3	34.3	36.6	974	719	32.1	28.7		
		35.3	35.3	36.1	970	719	32.1	26.6		
0.104	6.9-7.2	(see Table 1)							25.8	0.86
0.254	6.9	53.6	40.2	43.5	1067	729	30.8	20.7	22.5	0.72
		52.0	38.8	44.4	1060	729	30.8	22.4		
		37.2	37.2	34.2	978	720	30.4	22.9		
		36.6	36.6	34.6	975	720	30.4	23.9		
0.404	6.9	56.0	42.6	41.1	1064	733	29.8	17.7	20.2	0.62
		53.3	40.1	43.1	1059	733	29.8	20.6		
		53.7	40.5	43.0	1063	727	29.6	20.1		
		37.4	37.4	33.5	971	719	29.2	22.0		
		38.2	38.2	32.7	970	719	29.2	20.6		

there was no evidence of coenzyme instability, in that the extinction at 340 mμ remained constant after equilibrium was reached, it is possible that small losses of both coenzymes occur during the

approach to equilibrium, and that the coenzyme added initially is degraded to slightly greater extent.

The results of equilibrium measurements with

Fig. 1. Effect of ionic strength, I , on the equilibrium constants K_p (O) and K_c (Δ) of the isocitrate dehydrogenase reaction at 25°. K_p is defined by eqn. (2) and K_c by eqn. (4). The data are from Tables 1 and 2.

Fig. 2. Effect of temperature on the equilibrium constant, K_p , of the isocitrate dehydrogenase reaction, with I 0.104 (O) and I 0.254 (Δ).

other ionic strengths are shown in Table 2. The variation of K_p with ionic strength is small. If plots of $\log K_p$ against $\sqrt{I}$ (Fig. 1) are interpreted by the usual form of equation:

$$\log K_p = \log K_a + A\sqrt{I}$$

then the intercept gives a value for K_a at zero ionic strength and 25° of 30.9 atm., and the slope A is -0.26 . A plot of $\log K_c$ against $\sqrt{I}$ is also shown in Fig. 1.

Equilibrium measurements with I 0.104 were made at three other temperatures, and with I 0.254 at 37°. The equilibrium was approached from the NADP⁺ side. The results of these measurements, and those for 25° from Table 1, are shown as plots of $\log K_p$ against $1/T$ in Fig. 2. From the slope with I 0.104, the heat of reaction (1) is 13.75 kcal./mole. From this value, and the heat of solution of CO₂, -4.72 kcal./mole (Harned & Davis, 1943), ΔH for reaction (3) is 9.03 kcal./mole.

The effect on K_p of varying the concentration of magnesium sulphate in the reaction mixture, with

Fig. 3. Variation of the equilibrium constant of the isocitrate dehydrogenase reaction, K_p , with the logarithm of the concentration of MgSO₄ in the reaction mixture. Values for K_p , calculated from eqn. (2), were corrected to constant I 0.104 by means of the data of Fig. 1.

constant potassium hydrogen carbonate and potassium chloride concentrations, is shown in Fig. 3. The values of K_p were corrected to a constant ionic strength of 0.104 by means of the data of Fig. 1. There is evidently a considerable specific effect by high concentrations of magnesium sulphate on the equilibrium. For this reason the low concentration of 0.133 M, at which the specific effect is negligible, was used in all the experiments described above.

DISCUSSION

Ochoa (1948) obtained values for K_c of 0.51–1.02 M with different reactant concentrations and ionic strengths ranging from 0.02 to 0.12, at room temperature (21–23°). Large ratios of 2-oxoglutarate concentration to isocitrate concentration at equilibrium were needed to give accurately measurable ratios of oxidized to reduced coenzyme. The relatively small ionic-strength and temperature effects on K_c demonstrated here show that lack of control of these factors cannot account for the variability of Ochoa's (1948) data. A large excess of 2-oxoglutarate over isocitrate could also be a source of considerable error if even small amounts of isocitrate or oxalosuccinate were present in the 2-oxoglutarate. Despite the variability, the mean value of 0.77 M reported by Ochoa (1948) is in good agreement with that of 0.79 M for I 0.05 and 22° estimated from the results of the present experiments, in which larger carbon dioxide concentrations and smaller 2-oxoglutarate concentrations were used, and no significant variation of K_c with the reactant concentrations at constant ionic strength was observed.

Ochoa (1948) assumed that dissolved CO₂ is the product of the reaction. If the HCO₃⁻ ion were the

primary product, the true equilibrium constant of the isocitrate dehydrogenase reaction would be $K_c K'_1 / [\text{H}_2\text{O}]$, where:

$$K'_1 = \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{CO}_2] + [\text{H}_2\text{CO}_3]}$$

the apparent first ionization constant of carbonic acid. It has now been shown (Dalziel & Londesborough, 1968) that CO_2 is indeed the true substrate of isocitrate dehydrogenase (and of the 'malic' enzyme and 6-phosphogluconate dehydrogenase), and therefore eqn. (3) correctly describes the equilibrium, which is independent of the H^+ ion concentration. Strictly, K_c should be corrected for inclusion of H_2CO_3 in the dissolved CO_2 concentration, but the correction is negligible since only 0.1% of the total is hydrated.

The effect of ionic strength on the equilibrium is slight, compared with its effect on the glutamate dehydrogenase equilibrium, for example (Engel & Dalziel, 1967). This is to be expected if the effects can be interpreted in terms of changes of the activity coefficients of the ionic reactants in accordance with the Debye-Hückel limiting law:

$$\log \gamma = -0.5Z^2 \sqrt{I}$$

If the activity coefficients of the multivalent ions isocitrate³⁻, 2-oxoglutarate²⁻ and NADPH^{4-} are calculated from this relation, then from the experimental value of -0.26 for the slope of the plot of $\log K_p$ against $\sqrt{I}$ (Fig. 1), the activity coefficient of the zwitterion NADP^{+4-} is given by:

$$-\log \gamma = 5.76 \sqrt{I}$$

corresponding to an 'effective charge' of -3.4 . Although the application of the Debye-Hückel

limiting law to large multivalent ions is of uncertain validity, this result may have value as an empirical description.

The thermodynamic equilibrium constant for reaction (1), obtained by extrapolation of K_p to zero ionic strength, is 30.9 atm., from which $\Delta G^0 = -2.03$ kcal./mole. From this value and the standard free energy of solution of CO_2 , 2.00 kcal./mole (Burton & Wilson, 1953), ΔG^0 for reaction (3) is -0.03 kcal./mole. Also, from $\Delta G^0 = 5.93$ kcal./mole for the reduction of NADP^+ (Engel & Dalziel, 1967), the standard free-energy change for the oxidative decarboxylation of isocitrate:

is -7.96 kcal./mole. Burton & Wilson (1953) calculated a value for ΔG^0 of -12.69 kcal./mole for this reaction from Ochoa's (1948) value for K_c at $I \sim 0.05$, which was incorrectly reported as $0.77 \times 10^4 \text{M}$ (cf. Harary, Korey & Ochoa, 1953). Related free-energy data derived by Burton & Krebs (1953) contain the same error. This was corrected by Burton (1957), who calculated $\Delta G' = 2.32$ kcal./mole, corresponding to $\Delta G^0 = 7.23$ kcal./mole, for reaction (6). A value of 5.44 kcal./mole for the free energy of reduction of NADP^+ (Burton & Wilson, 1953) was used in this calculation, which accounts for part of the discrepancy with the present value.

From $\Delta G^0 = -7.96$ kcal./mole for reaction (6), and the standard free energy of reduction of NAD^+ , 5.22 kcal./mole (Burton & Wilson, 1953), ΔG^0 for the oxidative decarboxylation of isocitrate by NAD^+ is -2.74 kcal./mole.

These and other free-energy data are summarized in Table 3. ΔG^0 values refer to the usual standard

Table 3. Free-energy data for the isocitrate dehydrogenase reaction

The data are calculated from the equilibrium constant, K_p , for the NADP^+ -linked reaction measured in KHCO_3 - KCl solution of various concentrations and extrapolated to zero ionic strength. ΔG^0 values are for unit activity of all reactants in aqueous solution at 25° , except that CO_2 is the gas at 1 atm. $\Delta G'$ and E'_0 refer to a standard state of the H^+ ion of pH 7.0, and $\Delta G''$ and E''_0 refer in addition to a standard state for CO_2 of 0.05 atm. The calculation of these quantities from ΔG^0 is described in the text.

Reactants or oxidant (+ H_2 gas)	Products or reductant	ΔG^0 (kcal./mole)	$\Delta G'$ (kcal./mole)	$\Delta G''$ (kcal./mole)	E'_0 (v)	E''_0 (v)
Isocitrate ³⁻ + NADP^+	2-Oxoglutarate ²⁻ + $\text{NADPH} + \text{CO}_2$	-2.03	-2.03	-3.80	+0.044	+0.083
NADP^+	$\text{NADPH} + \text{H}^+$	+5.93*	-3.62	-3.62	-0.335	-0.335
2-Oxoglutarate ²⁻ + CO_2	Isocitrate ³⁻ + H^+	+7.96	-1.59	+0.18	-0.379	-0.418
NAD^+	$\text{NADH} + \text{H}^+$	+5.22†	-4.33	-4.33	-0.320	-0.320
Isocitrate ³⁻ + NAD^+	2-Oxoglutarate ²⁻ + $\text{NADH} + \text{CO}_2$	-2.74	-2.74	-4.51	+0.059	+0.098

* Engel & Dalziel (1967).

† Burton & Wilson (1953).

states of unit activity of the ideal molar solution for dissolved reactants and 1 atm. pressure for CO₂. Values for $\Delta G'$ and E_0' are calculated for an arbitrary standard state of pH 7.0 for the H⁺ ion, and $\Delta G''$ and E_0'' for pH 7.0 and a standard state of CO₂ of 0.05 atm., which approximate physiological conditions. The relationships used to calculate these quantities from ΔG^0 , at 25° and with ΔG values in kcal./mole and E_0 values in volts, are:

$$\begin{aligned}\Delta G' &= \Delta G^0 - 2.303RT(\text{pH}) \\ &= \Delta G^0 - 9.55 \text{ (if H}^+ \text{ is a product)}\end{aligned}$$

$$\begin{aligned}\Delta G'' &= \Delta G' + 2.303RT \log 0.05 \\ &= \Delta G' - 1.77 \text{ (if CO}_2 \text{ is a product)}\end{aligned}$$

$$\begin{aligned}E_0' &= -\frac{\Delta G'}{2F} - \frac{2.303RT(\text{pH})}{F} \\ &= -0.0217 \Delta G' - 0.414\end{aligned}$$

$$\begin{aligned}E_0'' &= E_0' - \frac{2.303RT \log 0.05}{2F} \\ &= E_0' + 0.039\end{aligned}$$

The specific effect of increasing magnesium sulphate concentrations on the isocitrate dehydrogenase equilibrium (Fig. 3) can be explained by complex-formation with isocitrate and 2-oxoglutarate, and indicates that the Mg²⁺ ion has greater affinity for isocitrate than for 2-oxoglutarate. Unfortunately, the stability constants for these Mg²⁺ ion complexes do not appear to be recorded, but those for the Ca²⁺ and Sr²⁺ ion complexes of isocitrate are greater than those for 2-oxoglutarate (Sillen & Martell, 1964). The effect of formation of complexes of the substrates with Mg²⁺ ions on the equilibrium constant of the isocitrate dehydrogenase reaction should be described by:

$$K_{\text{app.}} = \frac{K(1 + K_1[\text{Mg}^{2+}])}{(1 + K_2[\text{Mg}^{2+}])}$$

where K is the true equilibrium constant in the absence of Mg²⁺ ions, and K_1 and K_2 are the stability constants of the 2-oxoglutarate and isocitrate complexes respectively. The experimental results of Fig. 3 can be fairly well described by this equation if values of $K_1 = 60 \text{ M}^{-1}$ and $K_2 = 300 \text{ M}^{-1}$ are assumed. Although the data will not bear any firm conclusions, these values are reasonable in comparison with the available stability constants

for complexes of bivalent metal ions with these and related acids (Sillen & Martell, 1964).

At 37° and with I 0.25, which approximates conditions in liver tissue (Hohorst, 1960; Williamson, Lund & Krebs, 1967), the values obtained for K_p and K_c are 48.9 atm. and 1.17 M respectively. The values were obtained in the presence of 0.133 mM-Mg²⁺ ion and would be smaller in the presence of larger concentrations of bivalent metal ion. Mg²⁺ ion is the predominant bivalent metal ion in the soft tissues, and its total concentration in most tissues is 10–20 mM (Widdowson & Dickerson, 1964; Wacker & Vallee, 1964). The concentration of free Mg²⁺ ions available for complex-formation with isocitrate and 2-oxoglutarate may be very small, however.

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