31. *The Ionisation Constant of the Protonated Form of Creatinine.*

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Accurate measurements have been made of the acid dissociation constants of the creatininium ion at 10°, 20°, 30°, 40°, and 50° by a spectrophotometric procedure based on the difference in the absorption of the protonated and the non-protonated form of creatinine at the absorption maximum of the latter (234 mμ). The dissociation constants were also determined at 15°, 25°, 35°, 45°, and 55° by an e.m.f. method, by means of cells without liquid junction, of the type, Pt,H_2 | creatinine HCl | AgCl;Ag. The two sets of results show satisfactory agreement. The following equations in temperature have been fitted to both sets by least squares:

$pK = 8{\cdot}3717 - 0{\cdot}011884T$; and $pK = 198{\cdot}233/T + 7{\cdot}0705 - 0{\cdot}009753T$.

The significance of the results is discussed.

The ionisation of creatinine is interesting because of its biological importance and because of its unusual behaviour in solution where it undergoes hydrolysis to give an equilibrium mixture with creatine. In consequence of the relatively high solubility of the base and the

change in its ultraviolet absorption spectrum on protonation, it is possible to measure its dissociation constant by both spectrophotometric and e.m.f. methods. However, in each case a special technique has to be developed to obviate the difficulties associated with the change in concentration as a result of the hydrolysis.

Methods

Spectrophotometric Method.—The ionisation constant of creatinine can be determined spectrophotometrically since the unprotonated species has a pronounced absorption maximum at 234 mμ, which is abolished in the presence of excess acid.[1-3] The molar extinction coefficients of the protonated (ε_1) and unprotonated (ε_2) creatinine species were determined from optical-density measurements on solutions containing various concentrations of creatinine in N-hydrochloric acid and 0·01M-borax, respectively.

Five solutions were used in each series with $\sim 1 \times 10^{-5}$ to 2×10^{-4}M creatinine concentrations (c_1, c_2, . . . c_5). The solutions were made up by weight, and their densities at room temperature were determined to correct the concentrations to the molar scale. Corrections were applied to the molar concentrations at other temperatures.

Optical-density measurements were made at 10°, 20°, 30°, 40°, and 50° with a Hilger "Uvispek" photoelectric spectrophotometer, fitted with a cell compartment through which fluid at a constant temperature was circulated.

The weakest solution (c_1) was used as a blank in each series. The observed optical density of some solution (c_x) is given by $D_x = \varepsilon c_x + A$. Since, when the creatinine concentration is equal to that of the blank, $D_1 = \varepsilon c_1 + A = 0$, it follows that $D_x = \varepsilon(c_x - c_1)$. Application of the method of least squares to the results at each temperature gave the extinction coefficients.

The extinction coefficients show a slight variation with temperature: those for the protonated species (ε_1) decrease, and those for the unprotonated species (ε_2) increase, with increasing temperature. These contrary temperature effects suggest that the changes are real and not due to the effects of temperature on the measuring system. Equations in temperature were fitted to the values of ε_1 and ε_2 obtained, namely:

$$\varepsilon_1 = (1555 - 2{\cdot}0t) \pm 56$$
$$\varepsilon_2 = (6820 + 3{\cdot}7t) \pm 17.$$

These smoothed values were used in the subsequent calculations.

The following procedure was used in the determination of the ionisation constant of creatinine. A stock chloride-containing acetate solution was prepared, by weight, from acetic acid, potassium hydroxide solution, potassium chloride, and water. A stock solution of creatinine hydrochloride was also prepared by weight. A series of eight experimental solutions were then prepared by weighing out suitable amounts of these two stock solutions and adding water. The molal concentrations of acetic acid (m_1), potassium hydroxide (m_2), potassium chloride (m_3), and creatinine hydrochloride (m_4) could therefore be calculated for each solution. The densities of these solutions were determined at room temperature so as to calculate the total molar concentrations of creatinine of all species (c_{RT}). Corrections were applied for other temperatures.

The degree of dissociation (α) of the creatininium ion in these solutions was determined from optical-density measurements at 234 mμ at 10°, 20°, 30°, 40°, and 50° by using the following relation:

$$\frac{\alpha}{1-\alpha} = \frac{D - \varepsilon_1 c_{RT}}{\varepsilon_2 c_{RT} - D} = \frac{m_R}{m_{HR^+}},$$

where m_R and m_{HR^+} are the molal concentrations of the unprotonated and the protonated creatinine species, respectively. As can be seen from the above, the molar concentration of creatinine is required solely for the calculation of α from the optical densities and the dissociation constant is, in fact, finally obtained on the molal scale.

The molal concentration of hydrogen ions in solution is calculated as follows. The apparent

[1] Grinbaum and Marchlewski, *Bull. Internat. Acad. Polon., Classe Sci. Math. Nat. Sér. A*, 1937, 156.
[2] Ratner, Petrack, and Rochovansky, *J. Biol. Chem.*, 1953, **204**, 95.
[3] Wollenberger, *Acta Chem. Scand.*, 1953, **7**, 445.

dissociation constant of acetic acid, $K_{Ac}' = m_{H^+}m_{Ac^-}/m_{HAc}$, was evaluated from the thermodynamic data of Harned and Ehlers.[4] By introduction of the activity coefficient, we obtain

$$pK_{Ac}' = pK_{Ac} + 2 \log \gamma;$$

γ was assumed equal to the mean activity coefficient of hydrochloric acid.[5] Let a and b be the molal concentrations of free acetic acid and acetate ions, respectively, calculated for the solution from the amount of stock taken. Since the acetate is present as the potassium salt, we have from the electroneutrality condition

$$m_{K^+} + m_{H^+} + m_{HR^+} = m_{Cl^-} + m_{Ac^-} + m_{OH^-},$$

where the contribution of KCl to m_{K^+} and m_{Cl^-} has been ignored. $m_{K^+} = b$ and $m_{Cl^-} = m_{R_T}$; therefore, ignoring m_{OH^-} and m_{H^+}, we obtain $m_{Ac^-} = b + m_{HR^+} - m_{R_T} = b - m_R$. Thus, since the total concentration of all the acetic acid species is known, we can find m_{HAc}, and hence m_{H^+} also. We can now calculate the concentration dissociation constant of the creatininium ion: $K_{cr}' = m_{H^+}m_R/m_{HR^+}$. By plotting pK_{cr}' against I we can, in principle, extrapolate the results to zero ionic strength and obtain the thermodynamic constant. However, owing to small errors in the values of the optical densities there is considerable scatter about these plots of pK_{cr}', which causes large errors in the intercepts. To eliminate this effect as much as possible, the observed optical-density readings for each solution were fitted by least squares to the equation

$$D = \alpha + \beta t + \gamma t^2, \qquad (1)$$

where t is the temperature in degrees Celsius. The smoothed values of D calculated from the constants α, β, and γ were used in the evaluation of pK_{cr}'.

The thermodynamic dissociation constant of the creatininium ion can be represented by the expression:

$$pK_{cr}' = pK_{cr} + \log \gamma_{H^+}\gamma_R/\gamma_{HR^+}.$$

If the ion-size parameters in Debye–Hückel equations for the activity coefficients of H^+ and HR^+ were similar, the non-linear parts of the equations would cancel, and pK_{cr}' would be a linear function of I (on the assumption that γ_R does not vary much with I). However, this assumption is not usually justified and we therefore introduce the term $CI^{3/2}$ to allow for the non-linear variation, *i.e.*, the smoothed concentration constants are fitted to the following equation in I:[6]

$$pK_{cr} = pK_{cr}' - BI - CI^{3/2}. \qquad (2)$$

E.M.F. Method.—Cells without liquid junction of the following type were used:

$$Pt,H_2\ (1\ atm.)|KCl\ (m_3),\ creatinine\ HCl\ (m_4),\ Na_2HPO_4\ (m_5)|AgCl,Ag$$

where m_3, m_4, and m_5 are molal concentrations; both electrodes were in the same compartment. Since creatinine undergoes hydrolysis in solution to creatine, there is a drift in the e.m.f. of such a cell and its true value cannot be obtained. Consequently the cell was initially filled with a solution containing disodium hydrogen phosphate and potassium chloride. After the e.m.f. had become steady, solid creatinine hydrochloride was added and rapidly dissolved by stirring with a current of hydrogen. The e.m.f. was then read at timed intervals and extrapolated back to the point in time at which the solid creatinine had been added; this was taken as the e.m.f. of the cell. After the equilibration (40—50 min.), the variation of the e.m.f. of the cell with time became linear (Fig. 1). Presumably the initial disturbance of the e.m.f. is due to the necessity to resaturate the solution with hydrogen and for the silver–silver chloride electrode to come into equilibrium with the new chloride concentration. Linear equations were fitted by least squares to the variation of the e.m.f. with time from ~40 to ~100 min. after the addition of creatinine. From these equations the e.m.f. at zero time was calculated. Disodium hydrogen phosphate was used as the alkali in these buffers because the cell does not operate well at the high pH's, which would have obtained had an alkali-metal hydroxide been used. Unfortunately

[4] Harned and Ehlers, *J. Amer. Chem. Soc.*, 1932, **54**, 1350; 1933, **55**, 652.
[5] Bates and Bower, *J. Res. Nat. Bur. Stand.*, 1954, **53**, 283.
[6] Datta and Grzybowski, *Trans. Faraday Soc.*, 1958, **54**, 1179.

at pH values near the pK of creatinine, proton transfer to the monohydrogen phosphate anion is not complete and an approximate correction has to be made.

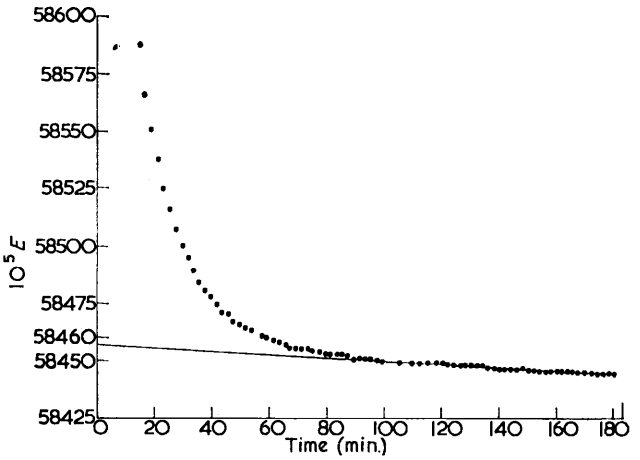

FIG. 1. Change in the e.m.f. at 25° of a cell Pt,H_2|KCl, creatinine HCl, Na_2HPO_4|Ag;AgCl after addition of solid creatinine hydrochloride at time 0 to the cell which contained a solution of KCl and Na_2HPO_4 only. The line represents the extrapolation of the linear part of the plot (from 91 to 180 min.) to obtain the true e.m.f. of the complete cell at time 0.

The following equilibria have to be considered:

$$K_{ph_1} = m_{H_2A^-} m_{H^+} / m_{H_3A}, \tag{3}$$

$$K_{ph_2} = m_{HA^{2-}} m_{H^+} / m_{H_2A^-}, \tag{4}$$

$$K_{cr} = m_R m_{H^+} / m_{HR^+}, \tag{5}$$

where m_{H_3A} is the concentration of phosphoric acid, and $m_{H_2A^-}$ and $m_{HA^{2-}}$ are the concentrations of the phosphate mono- and di-anions. The conservation equations are:

$$m_{A_T} = m_{HA^{2-}} + m_{H_2A^-} + m_{H_3A}, \tag{6}$$

where m_{A_T} is the overall concentration of all phosphoric acid and phosphate species, and

$$m_{R_T} = m_R + m_{HR^+}. \tag{7}$$

Ignoring the contributions by potassium chloride and the hydroxide ion, we have the electroneutrality condition:

$$m_{Na^+} + m_{HR^+} + m_{H^+} = 2m_{HA^{2-}} + m_{H_2A^-} + m_{Cl^-}. \tag{8}$$

Since $m_{Na^+} = 2m_{A_T}$ and $m_{Cl^-} = m_{R_T}$, from eqns. (6), (7), and (8) we have:

$$m_R = m_{A_T} + m_{H^+} - m_{HA^{2-}} + m_{H_3A}. \tag{9}$$

From eqns. (4) and (6), we have:

$$m_{HA^{2-}} = \frac{K_{ph_2}(m_{A_T} - m_{H_2A^-})}{K_{ph_2} + m_{H^+}}. \tag{10}$$

Similarly from eqns. (3) and (6), we have:

$$m_{H_3A} = \frac{m_{H^+}(m_{A_T} - m_{HA^{2-}})}{K_{ph_1} + m_{H^+}}. \tag{11}$$

Therefore:

$$m_{\mathrm{HA}^{\pm}} - m_{\mathrm{H_2A}} = \frac{K_{\mathrm{ph_2}} m_{\mathrm{A_T}}}{K_{\mathrm{ph_2}} + m_{\mathrm{H^+}}} - \left[\frac{m_{\mathrm{H^+}}(m_{\mathrm{A_T}} - m_{\mathrm{HA^{\pm}}})}{K_{\mathrm{ph_1}} + m_{\mathrm{H^+}}}\right]\left(\frac{2K_{\mathrm{ph_2}} + m_{\mathrm{H^+}}}{K_{\mathrm{ph_2}} + m_{\mathrm{H^+}}}\right) \quad (12)$$

Since $m_{\mathrm{HA^{\pm}}} < m_{\mathrm{A_T}}$ and $m_{\mathrm{H^+}} > K_{\mathrm{ph_2}}$, we have:

$$m_{\mathrm{HA^{\pm}}} - m_{\mathrm{H_2A}} = \frac{K_{\mathrm{ph_2}} \cdot m_{\mathrm{A_T}}}{K_{\mathrm{ph_2}} + m_{\mathrm{A_T}}} - \frac{m_{\mathrm{H^+}} \cdot m_{\mathrm{A_T}}}{K_{\mathrm{ph_1}} + m_{\mathrm{H^+}}} = Q. \quad (13)$$

From eqns. (7), (9), and (13) we have:

$$m_{\mathrm{R}} = m_{\mathrm{A_T}} + m_{\mathrm{H^+}} - Q$$

and

$$m_{\mathrm{HR^+}} = m_{\mathrm{R_T}} - m_{\mathrm{A_T}} - m_{\mathrm{H^+}} + Q.$$

Putting $m_{\mathrm{R_T}} = m_4$, $m_{\mathrm{A_T}} = m_5$, and $m_{\mathrm{KCl}} = m_3$, we have

$$\mathrm{p}K_{\mathrm{cr}} = \frac{(E - E^{\circ})\boldsymbol{F}}{\boldsymbol{RT} \ln 10} + \log \frac{m_4 - m_5 - m_{\mathrm{H^+}} + Q}{m_5 + m_{\mathrm{H^+}} - Q} + \log (m_3 + m_4) - 2\boldsymbol{A}I^{\frac{1}{2}} - \boldsymbol{B}I - \boldsymbol{C}I^{3/2}, \quad (14)$$

where $(m_3 + m_4)$ is the total chloride ion concentration and $2\boldsymbol{A}I^{\frac{1}{2}} + \boldsymbol{B}I + \boldsymbol{C}I^{3/2} = -\log \gamma_{\mathrm{HR^+}}\gamma_{\mathrm{Cl^-}}$;[6] here $\boldsymbol{A}$ is the Debye–Hückel slope and $\boldsymbol{B}$ and $\boldsymbol{C}$ are arbitrary parameters.

The ionic strength is given by:

$$\begin{aligned} I &= \tfrac{1}{2}(m_{\mathrm{Cl^-}} + m_{\mathrm{Na^+}} + m_{\mathrm{K^+}} + m_{\mathrm{H_2A^-}} + 4m_{\mathrm{HA^{\pm}}} + m_{\mathrm{HR^+}} + m_{\mathrm{H^+}}) \\ &= m_3 + m_4 + m_5 + P, \end{aligned} \quad (15)$$

where

$$P = \frac{2K_{\mathrm{ph_2}} \cdot m_5}{K_{\mathrm{ph_2}} + m_{\mathrm{H^+}}} - \frac{m_{\mathrm{H^+}} \cdot m_5}{K_{\mathrm{ph_1}} + m_{\mathrm{H^+}}}. \quad (16)$$

Initially an approximate value for the ionic strength $I' = m_3 + m_4 + m_5$ is used to find $m_{\mathrm{H^+}}$ from the e.m.f. of the cell and the molality of the chloride ion:

$$-\log m_{\mathrm{H^+}} = \frac{(E - E^{\circ})\boldsymbol{F}}{\boldsymbol{RT} \ln 10} + \log (m_3 + m_4) + 2 \log \gamma_{\pm},$$

where $\gamma_{\pm}$ is the mean activity coefficient of hydrogen chloride. Values of the quantities P and Q may then be calculated and used to evaluate the buffer ratio and the ionic strength in eqns. (14) and (15).

Let

$$y = \frac{(E - E^{\circ})\boldsymbol{F}}{\boldsymbol{RT} \ln 10} + \log \frac{m_4 - m_5 - m_{\mathrm{H^+}} + Q}{m_5 + m_{\mathrm{H^+}} - Q} + \log (m_3 + m_4) - 2\boldsymbol{A}I^{\frac{1}{2}}; \quad (17)$$

then $\mathrm{p}K_{\mathrm{cr}} = y - \boldsymbol{B}I - \boldsymbol{C}I^{3/2}$, which can be fitted by least squares to give $\mathrm{p}K_{\mathrm{cr}}$.

Here again we have the problem of the undue error in the intercepts because of the scatter. To eliminate this, we proceed as follows. The initial values of y are fitted to the equation

$$y = \mathrm{p}K_{\mathrm{cr}} + \boldsymbol{B}I + \boldsymbol{C}I^{3/2}, \quad (18)$$

from which smoothed values of y at the various values of I are calculated. The set at each ionic strength is then fitted to the equation

$$y = \alpha + \beta t + \gamma t^2. \quad (19)$$

The values of y calculated from eqn (19) are finally refitted to eqn (18) and the thermodynamic pK's are obtained from the intercepts.

Results

The results of the spectrophotometric determinations are given in Table 1, and those of the e.m.f. determinations in Table 2. On plotting pK_{cr} against temperature, it was found that, unlike the case with most acids, the values lie on a straight line (Fig. 2). Therefore, in order to

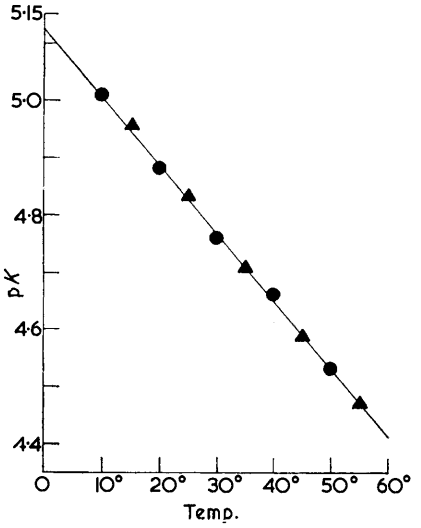

FIG. 2. Variation of the pK of creatinine with temperature.
● By spectrophotometric method.
▲ By e.m.f. method.
The line represents the equation $pK = 8{\cdot}3717 - 0{\cdot}011884T$.

TABLE 1.

Molalities and optical densities of solutions and values of pK_{cr}'.

m_1 = molality of acetic acid; m_2 = molality of KOH; m_3 = molality of KCl; m_4 = molality of creatinine HCl. D is the observed optical density at 234 mμ (average of 3 or more readings); the numbers in parentheses are the deviations in the last figures of the observed values from the lines of eqn. (1). $I = m_2 + m_3 + m_4$.

	10^2m_1	1·82304	2·89217	3·91221	4·98830	6·02885	7·19497	8·16970	9·14567
	10^2m_2	1·32742	2·10590	2·84863	3·63221	4·38984	5·23893	5·94867	6·65931
	10^2m_3	0·73489	1·16588	1·57707	2·01086	2·43033	2·90041	3·29334	3·68676
	10^4m_4	2·97413	2·99647	2·99980	2·99095⁻	3·00974	2·99677	3·01653	2·99519
10°	D	1·293(−2)	1·290(0)	1·293(−1)	1·286(−1)	1·298(+2)	1·303(+3)	1·322(−1)	1·310(−1)
	pK_{cr}'	$5{\cdot}003_7$	$5{\cdot}005_4$	$4{\cdot}989_3$	$4{\cdot}982_7$	$4{\cdot}971_7$	$4{\cdot}952_1$	$4{\cdot}831_1$	$4{\cdot}929_2$
20°	D	1·418(+5)	1·417(0)	1·420(+2)	1·417(0)	1·430(−3)	1·425(−5)	1·445(+2)	1·443(+3)
	pK_{cr}'	$4{\cdot}862_0$	$4{\cdot}855_1$	$4{\cdot}843_7$	$4{\cdot}828_4$	$4{\cdot}810_9$	$4{\cdot}800_0$	$4{\cdot}788_2$	$4{\cdot}773_2$
30°	D	1·516(−5)	1·531(−1)	1·537(+3)	1·538(+4)	1·555(0)	1·544(−3)	1·560(−2)	1·561(−2)
	pK_{cr}'	$4{\cdot}733_6$	$4{\cdot}720_2$	$4{\cdot}706_4$	$4{\cdot}689_1$	$4{\cdot}666_2$	$4{\cdot}658_1$	$4{\cdot}644_3$	$4{\cdot}622_7$
40°	D	1·622(+2)	1·634(+1)	1·636(−5)	1·633(−5)	1·663(+2)	1·663(+8)	1·682(+1)	1·679(+1)
	pK_{cr}'	$4{\cdot}614_3$	$4{\cdot}599_2$	$4{\cdot}577_7$	$4{\cdot}563_6$	$4{\cdot}536_5$	$4{\cdot}624_3$	$4{\cdot}494_8$	$4{\cdot}474_3$
50°	D	1·710(0)	1·722(0)	1·743(+3)	1·731(+2)	1·750(−1)	1·750⁻(−4)	—	—
	pK_{cr}'	$4{\cdot}496_5$	$4{\cdot}483_0$	$4{\cdot}442_7$	$4{\cdot}440_6$	$4{\cdot}410_7$	$4{\cdot}383_5$	—	—

compare the two sets of measurements, which were done at different temperatures, the following equation relating pK_{cr} to temperature was fitted by least squares to all the data taken together:

$$pK_{cr} = 8{\cdot}3717 - 0{\cdot}011884T \qquad (20)$$

The thermodynamic quantities associated with the ionisation of the creatininium ion were calculated from its parameters in the usual way (Table 3). From the results given in Table 3, it is evident that the two sets of measurements are in good agreement. This is very satisfactory in view of the very different nature of the two methods employed in this work.

TABLE 2.

Molalities and ionic strengths [eqn. (15)] of solutions, e.m.f.s of the cell:

Pt,H_2(1 atm.) | KCl(m_3), creatinine HCl(m_4), Na_2HPO_4(m_5) | AgCl; Ag (abs. v), and extrapolation functions y [eqn. (17)].

15°	$10^3 m_3$	3·9636	5·1269	6·3989	7·3731	8·6592	10·0295	11·0185	12·2272
	$10^2 m_4$	1·24226	1·41815	1·92896	2·17335	2·61643	2·77779	3·26557	3·4592
	$10^3 m_5$	3·9563	5·1174	6·3871	7·3594	8·6431	10·0109	10·9981	12·2044
	$10^2 I$	2·03665	2·44703	3·21257	3·65296	4·35424	4·79278	5·47852	5·89311
	$10^5 (E - E°)$	37,274	37,384	36,424	36,234	35,756	35,912	35,391	35,414
	y	4·9185⁻	4·9154	4·9055⁻	4·9006	4·8936	4·8899	4·8909	4·8796
25°	$10^3 m_3$	3·8257	4·8131	6·0058	6·9807	8·6521	9·1256	10·2418	11·4870
	$10^2 m_4$	1·23644	1·53230	1·94085	2·24611	2·59849	2·95673	3·33379	3·67724
	$10^3 m_5$	3·7699	4·7428	5·9181	6·8788	8·5644	8·9924	10·0923	11·3193
	$10^2 I$	1·99713	2·48948	3·13542	3·64045	4·32493	4·77292	5·37243	5·96445
	$10^5 (E - E°)$	37,776	37,262	36,688	36,386	36,237	35,766	35,486	$35,324_7$
	y	4·8030	4·7858	4·7804	4·7773	4·7596	4·7681	4·7621	4·7565
35°	$10^3 m_3$	3·8132	$4·9324_6$	6·0099	7·1726	8·1939	9·3315	10·3823	11·4371
	$10^2 m_4$	1·22147	1·56169	1·93981	2·20840	2·46857	2·92263	3·29426	3·63234
	$10^3 m_5$	3·7575	$4·8604_5$	5·9222	7·0679	8·0740	9·1953	10·2307	11·2702
	$10^2 I$	1·97853	2·54128	3·13347	3·63378	4·09743	4·77751	5·35812	5·90623
	$10^5 (E - E°)$	38,340	37,797	37,224	37,091	36,909	36,402	36,090	35,870
	y	4·6730	4·6673	4·6604	4·6574	4·6529	4·6475⁻	4·6421	4·6374
45°	$10^3 m_3$	3·7734	4·9408	5·9499	6·9467	8·1736	9·2753	10·3692	11·6482
	$10^2 m_4$	1·22570	1·51158	1·87923	2·26739	2·52675	2·88981	3·24415	3·55269
	$10^3 m_5$	3·7192	4·8687	5·8630	6·8453	8·0543	9·1399	10·1178	11·4782
	$10^2 I$	1·97367	2·49153	3·05928	3·64481	4·14874	4·73061	5·30231	5·86560
	$10^5 (E - E°)$	$38,894_5$	38,574	37,952	37,798	37,331	36,990	36,707	36,591
	y	4·5660	4·5596	4·5554	4·5500⁻	4·5461	4·5403	4·5413	4·5306
55°	$10^3 m_3$	4·0270	5·1681	6·2868	7·4764	8·6822	9·8839	11·0736	12·2331
	$10^2 m_4$	1·22418	1·42388	1·92218	2·14474	2·61024	2·91356	3·30090	3·26563
	$10^3 m_5$	4·0195	5·1585	6·2752	7·4626	8·6660	9·8655	11·0530	12·2103
	$10^2 I$	2·02645	2·45460	3·17512	3·63599	4·34152	4·88519	5·50974	5·70865
	$10^5 (E - E°)$	39,794	39,759	38,641	38,632	37,968	37,790	37,406	37,878
	y	4·4656	4·4600	4·4528	4·4527	4·4431	4·4401	4·4270	4·4303

TABLE 3.

Values of pK of creatinine and the thermodynamic quantities for the acid dissociation.

pK_{obs} was obtained spectrophotometrically at 10°, 20°, 30°, 40°, and 50° from eqn. (2) and by e.m.f. at 15°, 25°, 35°, 45°, and 55° from eqn. (18). The thermodynamic quantities were calculated from $pK_{calc} = 8·3717 - 0·0118847$. The errors were calculated from $V(pK)$ by the method of Please [9] adapted for a linear equation in T.

Temp.	pK_{obs}	pK_{calc}	$\Delta G°$ (kJ mole^{-1})	$\Delta H°$ (kJ mole^{-1})	$-\Delta S°$ (J mole^{-1} deg.$^{-1}$)	$\Delta C_p°$ (J mole^{-1} deg.$^{-1}$)
10° *	5·009	5·007	27·14 ± 0·02	18·24 ± 0·21	31·44 ± 0·68	128·8 ± 1·5⁻
15	4·954	4·947	27·29 ± 0·02	18·89 ± 0·22	29·16 ± 0·71	131·1 ± 1·5
20 *	4·881	4·888	27·43 ± 0·02	19·55 ± 0·22	26·88 ± 0·73	133·4 ± 1·5
25	4·829	4·829	27·56 ± 0·01	20·22 ± 0·23	24·61 ± 0·76	135·7 ± 1·6
30 *	4·760	4·769	27·68 ± 0·01	20·91 ± 0·24	22·33 ± 0·79	137·9 ± 1·6
35	4·707	4·710	27·79 ± 0·01	21·60 ± 0·25⁻	20·06 ± 0·81	140·2 ± 1·6
40 *	4·661	4·650	27·88 ± 0·01	22·31 ± 0·26	17·78 ± 0·84	142·5⁻ ± 1·6
45	4·588	4·591	27·96 ± 0·02	23·03 ± 0·26	15·51 ± 0·86	144·8 ± 1·7
50 *	4·532	4·531	28·04 ± 0·02	23·76 ± 0·27	13·23 ± 0·89	147·0 ± 1·7
55	4·472	4·472	28·10 ± 0·02	24·50⁻ ± 0·28	10·96 ± 0·92	149·3 ± 1·7

$\sqrt{V(pK)} = 0·0062$.

* These results were obtained by spectrophotometry.

DISCUSSION

Creatinine can be represented by the following formulæ:

i.e., the extreme forms are 2-imino-1-methylimidazolidin-4-one or 2-amino-4-hydroxy-1-methylimidazole. More creatininium species are possible since the hydrogen can conceivably be located on any of the three nitrogen atoms, although perhaps the imino-nitrogen

and nitrogen-1 are more likely to be protonated than nitrogen-3 since in the imidazolidine form the latter has electronegative groups on either side. Wollenberger,[3] who studied the absorption of creatinine over a wide pH range, found that the protonated form also has an absorption maximum, but at a much lower wavelength (217 mμ; ε $4{\cdot}5 \times 10^3$). He interprets this hypsochromic shift as due to fixation of the distribution of the π-electrons by the positive charge conferred on the molecule in the acid medium. This would imply stabilisation of the iminoimidazolidinone form (or one of the intermediate forms). Dissociation might then be expected to result in the formation of appreciable amounts of the aminohydroxymethylimidazole form, which has a fixed planar structure. The process would therefore be associated with a decrease in entropy. This would be a so-called non-environmental entropy change, *i.e.*, largely independent of the medium and temperature: the ΔC_p° term associated with such a process should be zero. It is also possible, however, that removal of the positive charge would favour the so-called " iceberg effect," [7] which involves the formation of a quasi-crystalline region in the water, in the immediate vicinity of the creatinine molecule, which is associated with a high degree of order, so that the corresponding entropy change is negative. This process would be expected to involve a considerable positive ΔC_p° term (" melting of the iceberg ").

The values of pK_{cr} in the literature are based either on spectrophotometric or potentiometric titration data. As far as one can tell, most of them were obtained without allowance for the fairly rapid hydrolysis of creatinine in solution. Table 4 lists these pK's together with the conditions in which they were obtained.

TABLE 4.

Comparison of the pK's of creatinine obtained in this work and by other investigators.

Method *	Conditions	Temp.	pK	Ref.
A	0·02M	15°	4·91	*a*
C	$I = 0$	15	4·95	This work
A	$I \rightarrow 0$	20	4·85	*b*
C	$I = 0$	20	4·89	This work
A	0·1M	25	4·78	*a*
B	$I = 0{\cdot}002$—0·03	25	4·83	*c*
C	$I = 0$	25	4·83	This work
A	0·1M	30	4·77	*a*
C	$I = 0$	30	4·77	This work
B	$I = 0{\cdot}002$—0·03	40	4·53	*c*
C	$I = 0$	40	4·65	This work

* A = e.m.f. measurements on cells with hydrogen electrodes and liquid junctions during titration of creatinine with HCl. B = e.m.f. measurements on cells with hydrogen electrodes and liquid junctions containing creatinine hydrochloride solutions. C = Combined results from spectrophotometric and e.m.f. measurements on cells without liquid junction.

Refs.: (*a*) Cannan and Shore, *Biochem. J.*, 1928, **22**, 920. (*b*) Recalc. from Eadie and Hunter, *J. Biol. Chem.*, 1926, **67**, 237. (*c*) McNally, *J. Amer. Chem. Soc.*, 1926, **48**, 1003.

Wollenberger [3] noted that in alkaline solutions (0·9—1·5N-alkali) the form of the curve changes again, the absorption maximum becoming more intense and shifting to shorter wavelengths (225 mμ; ε $11{\cdot}1 \times 10^3$). On further increase in alkali concentration the maximum shifts back to longer wavelengths (229 mμ in 10N-KOH; 231·5 mμ in 16N-KOH). Wollenberger interprets these changes as due to successive ionisation of 1 and then 2 protons, to form a singly charged and a doubly charged anion. Creatine, the hydrolysis product of creatinine, absorbs very weakly in this region and does not exhibit comparable changes with pH.

The thermodynamic functions appear to support the " iceberg " hypothesis (high positive ΔC_p°, high negative ΔS° sharply decreasing with temperature). The errors in the thermodynamic quantities given in Table 3 were calculated from $V(pK) = 3{\cdot}8 \times 10^{-5}$, the variance about the line of eqn. (20). If, however, an equation such as (20) is fitted to

[7] Datta and Grzybowski, *Trans. Faraday Soc.*, 1958, **54**, 1188.

the values of pK_{cr} derived without the smoothing procedures described [eqns. (1) and (19)], $V(pK) = 13{\cdot}1 \times 10^{-5}$. This means that the smoothing procedures have nearly halved the calculated errors. The comparatively small errors in ΔC_p° are due to the use of a simple linear equation to describe the temperature dependence of pK_{cr}.

If an equation of the type suggested by Harned and Robinson [8] is fitted to the observed values we get:

$$pK_{cr} = 198{\cdot}233/T + 7{\cdot}0705 - 0{\cdot}009753T. \quad (21)$$

The fit of eqns. (20) and (21) to the experimental results is equally good. Thus $\Sigma\Delta^2 = 3{\cdot}067 \times 10^{-4}$ and $2{\cdot}866 \times 10^{-4}$ for eqns. (20) and (21), respectively, and $\sqrt{V(pK)} = \sqrt{\Sigma\Delta^2/(m-2)} = 0{\cdot}0062$ for eqn. (20) and $= \sqrt{\Sigma\Delta^2/(m-3)} = 0{\cdot}0064$ for eqn. (21). Since both equations describe the results accurately, the values of ΔG° are virtually the same when calculated from either. The other thermodynamic quantities show slight differences, particularly at the ends of the experimental temperature range. Thus at 10°, 30°, and 55°, the following differences are found: ΔH°[eqn. (20)] − ΔH°[eqn. (21)] = −0·53, −0·05, and +0·60 kJ mole^{-1}; ΔS°[eqn. (20)] − ΔS°[eqn. (21)] = −1·82, −0·18, and +1·86J mole^{-1} deg.$^{-1}$; and ΔC_p°[eqn. (20)] − ΔC_p°[eqn. (21)] = +23·1, +24·7, and +26·7J mole^{-1} deg.$^{-1}$. The errors in the thermodynamic quantities calculated by the methods of Please [9] are much larger (about 30 times) for ΔC_p derived from eqn. (21); the others are less affected by the nature of the temperature equation. This merely reflects the difficulty in fixing the true value of ΔC_p° from experiments of this type.

Experimental

Materials.—Creatinine hydrochloride. Commercial creatinine hydrochloride was recrystallised from bromine-free ~6N-hydrochloric acid and then from water. It was dried in a desiccator over P_2O_5. Its purity was checked by gravimetric determination of the chloride content (as silver chloride) and was found to be 99·76% and 99·66% for two different batches (each the average of three estimations).

Acetic acid. " AnalaR " material was redistilled and its concentration determined by pH titration with standard alkali.

Potassium hydroxide. A solution of carbonate-free potassium hydroxide was prepared electrolytically from " AnalaR " potassium hydroxide in a laboratory-scale Castner–Kellner cell. Its strength was determined by titration with standard hydrochloric acid.

Potassium chloride. " AnalaR " material was dissolved in water, the solution was chlorinated, and the solid was precipitated from solution by saturation with gaseous, bromine-free hydrogen chloride. The salt was filtered off, dried at 160°, and fused in an atmosphere of dry nitrogen.

Disodium hydrogen phosphate. " Anhydrous " " AnalaR " material was further dried at 110°. Its concentration in the stock solution was estimated by evaporating weighed amounts of the solution to dryness and weighing the resulting anhydrous salt; this material was then ignited and the resulting tetrasodium pyrophosphate weighed. The disodium hydrogen phosphate content of other samples of the stock solution was determined by precipitating the phosphate as magnesium hydrogen phosphate, igniting this salt to pyrophosphate, and weighing that. The combined results of these analyses (three of each) had a standard error of ±0·17%.

Hydrochloric acid. " AnalaR " concentrated acid was diluted to ~6N, chlorinated, boiled to drive off the excess of chlorine, and distilled. The concentration of hydrogen chloride in the resulting, constant-boiling acid was determined from the atmospheric pressure during the distillation. Standard solutions were prepared from this material by dilution.

Cells without Liquid Junction.—Each cell contained one hydrogen and one silver–silver chloride electrode in the same compartment. The phosphate–chloride solution (~200 g.) was added from a weight-burette. Hydrogen was passed through saturators containing another portion of the same phosphate–chloride solution. A weighed amount of creatinine hydrochloride was suspended in a glass container above the level of the solution in the electrode compartment;

[8] Harned and Robinson, *Trans. Faraday Soc.*, 1940, **36**, 973.
[9] Please, *Biochem. J.*, 1954, **56**, 196.

this container could be dropped into the solution without opening the cell. The electrodes were of the type previously described.[10]

Spectrophotometric Measurements.—The optical density of the solutions was measured in 1 cm. quartz cells in a Hilger " Uvispek " spectrophotometer, fitted with a jacketed cell-holder. At least three readings of the optical density were made on each solution and these were averaged to give the figures used in subsequent calculations. Kerosene, from a large thermostatically controlled bath, was pumped through the cell-holder. The temperature of the circulating kerosene was measured, with a platinum resistance thermometer, where it entered the spectrophotometer. The temperature in the cells was measured with a calibrated glass thermometer, and the kerosene temperature was adjusted until the cell contents were at the required temperature. The cell compartment was kept dry by silica gel; this was particularly necessary at low temperatures to prevent condensation of water on the optical components.

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[10] Ashby, Crook, and Datta, *Biochem. J.*, 1954, **56**, 190.