ters boiled at 168–170° (10 mm.). The hydrazide melted at 148–150° The acid of the black raspberry is therefore citric acid.

Concord Grape.—The esters of the acids from 19 kilograms of Concord grapes afforded the following results on fractionation at 10 mm.

Fraction	1	2	3	4	5	6	7
Boiling point, °C....	80–90	125–130	130–140	140–145	145–150	150–155	Residue
Weight, g..........	0.6	27.0	3.0	1.5	7.7	9.0	2.7
α_D.................	.....	−9.42	−6.37	−2.4	+7.0	+7.5	...
M. p. of hydrazide, °C..............	174–176	175–178	167–170	162–167	180–182	180–184	...

The hydrazides from Fractions 1 and 2 were shown, by mixed melting points, to be *l*-malic dihydrazide, those from Fraction 3 to be impure *l*-malic dihydrazide, those from Fraction 4 to be a mixture, and those from Fractions 5 and 6 to be *d*-tartaric dihydrazide.

The relative proportions of the two acids, calculated from the optical rotations of the fractions, were about 60% of *l*-malic acid and about 40% of *d*-tartaric acid.

Summary

By the ester-distillation method it was found that the acids of the strawberry were citric acid (about 90%) and *l*-malic acid (about 10%) and that the pineapple acids were citric acid (about 87%) and *l*-malic acid (about 13%); that in the red raspberry the acids were a mixture of citric acid (about 97%) with *l*-malic acid (about 3%); and in the black raspberry the acid was citric acid. The acids of the Concord grape were a mixture of *l*-malic acid (about 60%) and *d*-tartaric acid (about 40%).

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[CONTRIBUTION FROM THE COBB CHEMICAL LABORATORY OF THE UNIVERSITY OF VIRGINIA]

THE EQUILIBRIUM BETWEEN CREATINE AND CREATININE, IN AQUEOUS SOLUTION. THE EFFECT OF HYDROGEN ION

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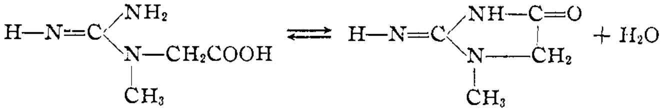
I. In Aqueous Solution

Introduction.—The reversible reaction



$$H{-}N{=}C\begin{cases}NH_2\\ N{-}CH_2COOH\\ \quad|\\ \ CH_3\end{cases} \rightleftharpoons H{-}N{=}C\begin{cases}NH{-}C{=}O\\ \quad\quad\ \ |\\ N{-}{-}CH_2\\ |\\ CH_3\end{cases} + H_2O \qquad (1)$$

Creatine Creatinine

is of interest not only from its importance in biochemistry, but also because of the ease with which it is adapted to physicochemical study. The literature contains numerous references to the conditions under which creatine is practically quantitatively converted to creatinine in acid solution. On the other hand, creatinine is at least partially converted into creatine in alkaline solution, the reaction having served as a method of preparation of creatine from creatinine.[1] Recent investigations have shown that the reaction from left to right in acid solution[2] and from right to left in alkaline solution[3] is essentially monomolecular. In neutral and alkaline solutions, a condition of equilibrium is reached,[3,4] in which appreciable quantities of both substances are present; in acid solution the position of equilibrium must lie so far on the right of Reaction 1 as to escape measurement. The scattered data occurring in the literature concerning the equilibrium are so unsatisfactory, and the exact effect of acid and alkali has been so obscure, that a careful determination of the equilibrium conditions, and the shift in the equilibrium concentrations produced by the hydrogen-ion concentration of the medium, has seemed desirable. Part I of the present investigation includes a study of the equilibrium in pure water at various temperatures. Part II will discuss the effect of hydrogen ion on the equilibrium.

Experimental Part

Materials.—Commercial creatine of very good quality was purified by two recrystallizations from water, with the addition of powdered charcoal. The product was pure white, odorless and finely crystalline. The solid thus prepared should contain one molecule of water of crystallization; to establish its purity, samples were heated to 100° and the loss in weight was determined. The results agree with the calculated value (12.04%) within the limits of experimental error.

Pure creatinine was prepared from creatine by the method of Edgar and Hinegardner.[5] Other materials were commercial C. P. products.

Analytical Methods.—For the determination of creatinine in the equilibrium mixtures, the colorimetric method of Folin[6] was employed, depending upon the red color produced by the reaction of alkaline picric acid solution upon creatinine. As analytical standards, pure creatinine and creatinine picrate[7] were employed. A Duboscq type of colorimeter was used for matching the colors, and all of the precautions necessary to obtain accurate results by this method were employed. Particular care was taken that the quantity of creatinine in the unknown solution and in the standard should be nearly the same, and that the treatment should be uniform.

Creatine was usually determined by difference, but in several cases, as a check, it was determined analytically through conversion into creatinine by evaporation to dryness with hydrochloric acid and subsequent colorimetric estimation.

Apparatus and General Technique.—Preliminary results showed that

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[1] Benedict, *J. Biol. Chem.*, **18**, 191 (1914).

[2] Edgar and Wakefield, This Journal, **45**, 2242 (1923).

[3] Hahn and Barkan, *Z. Biol.*, **72**, 305 (1920).

[4] Myers and Fine, *J. Biol. Chem.*, **21**, 583 (1915).

[5] Edgar and Hinegardner, *ibid.*, **56**, 881 (1923).

[6] Folin, *ibid.*, **17**, 463 (1914).

[7] Edgar, *ibid.*, **56**, 1 (1923).

the determination of the equilibrium between creatine and creatinine is complicated by the occurrence of side reactions (probably the hydrolysis to sarcosine, methylhydantoin, urea, etc.) when the attempt is made to allow a solution of either creatine or creatinine to come to equilibrium. The general method employed, therefore, consisted in making a preliminary determination of the equilibrium by preparing a number of mixtures containing creatine and creatinine in various proportions, heating them for given periods of time, and then determining by analysis the direction in which the reaction had proceeded in each case. After the preliminary experiment had approximately fixed the point of equilibrium, a new series of mixtures was made up corresponding to concentrations very close to this point, but on either side of it, and the solutions were again heated and analyzed. In this way the equilibrium concentrations could be determined with very considerable accuracy, without side reactions occurring to a measurable extent (except in one or two cases, as noted below). The solutions were contained in small flasks of Pyrex glass, which were sealed and immersed in a thermostat or, at 100°, in boiling water. At the end of appropriate time intervals the flasks were cooled rapidly, opened and the contents analyzed.

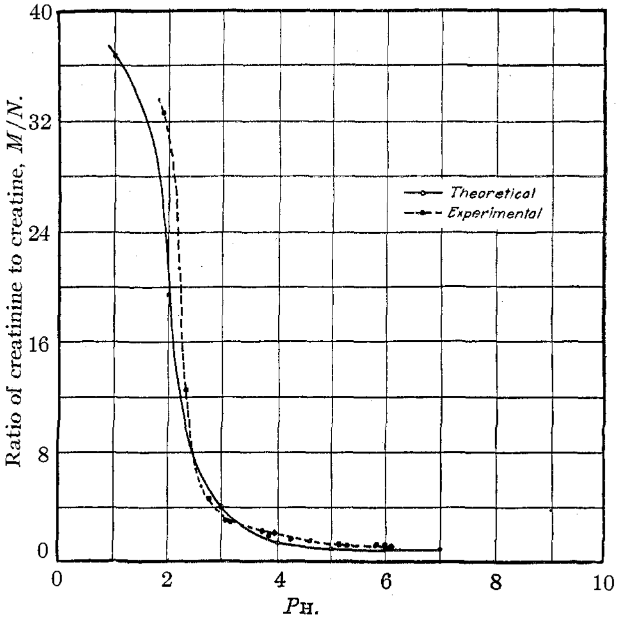

Fig. 1.—Effect of hydrogen-ion concentration on the creatine-creatinine equilibrium.

Experimental Data

A characteristic experiment at 50°, giving both preliminary and final results, is shown in Table I. Similar data, obtained at 25°, 70° and 100°

are given in Tables II, III and IV. The preliminary experiments at 25° and 100° are omitted, as the approximate equilibrium conditions could be estimated from the data at 50° and 70°. From the equilibrium concentrations the equilibrium constants

$$K = \frac{\text{Concentration of creatinine}}{\text{Concentration of creatine}} \qquad (2)$$

have been calculated. The total concentrations were 0.01 M in all cases.

Table I

Results at 50°

Time, 150 hours

Mole per cent. added		Mole per cent. found
Creatine	Preliminary Creatinine	Creatinine
60	40	48.01
55	45	49.17
50	50	50.36
45	55	52.21
40	60	55.25
	Final. K = 1.02	
51	49	50.18
50	50	50.37
49	51	50.72
48	52	51.16
47	53	51.69

Table II

Results at 70°

Time, 70 hours

Mole per cent. added		Mole per cent. found
Creatine	Preliminary Creatinine	Creatinine
45	55	...
40	60	61.43
35	65	62.26
30	70	63.13
25	75	64.01
	Final. K = 1.59	
40	60	61.00
39	61	61.34
38	62	61.52
37	63	61.89
36	64	62.29

Table III

Results at 25°

Time, 2460 hours. K = 0.540

Mole per cent. added		Mole per cent. found	
Creatine	Creatinine	Creatinine	Total
67	33	31.39	89.72
66	34	...	...
65	35	31.31	88.51
64	36	31.27	88.02
63	37	31.44	91.29

Table IV

Results at 100°

Time 4 hours. K = 2.89

Mole per cent. added		Mole per cent. found
Creatine	Creatinine	Creatinine
28	72	...
27	73	73.74
26	74	74.11
25	75	74.50
24	76	74.89

It will be noted that in the experiments at 25° about 10% of the total creatine and creatinine had decomposed through side reactions in the three months' time of the experiments. At other temperatures no evidence of measurable decomposition could be obtained.

Discussion of Data

It is evident from the data that Reaction 1 does proceed definitely to a condition of equilibrium in aqueous solution, and that the equilibrium is markedly affected by temperature, the proportion of creatinine increasing with increasing temperature. The statement occurring occasionally in biochemical literature that creatine can be converted quantitatively to creatinine by continued boiling with water alone is, however, obviously incorrect.

It will be shown in the second part of this paper that the equilibrium is very sensitive to changes in hydrogen-ion concentration for certain values of P_H, but that this is not the case for the faintly alkaline solutions obtained by dissolving creatine and creatinine in pure water. The results obtained above correspond, therefore, to the correct values for the equilibrium constant for Equation 1 as written.

From the values of the equilibrium constants at the four temperatures employed, three values of the increase in heat content may be calculated by the van't Hoff equation,

$$\frac{\mathrm{d}\,ln\,K}{\mathrm{d}T} = \frac{\Delta H}{RT^2} \tag{3}$$

The data thus obtained show concordant results, the mean of which is 4963 calories, for the heat absorbed in the reaction. If we write the integrated form of Equation 3 we obtain,

$$ln\,K = \frac{-\Delta H}{T} + C \tag{4}$$

or converting to ordinary logarithms,

$$\log K = \frac{-\Delta H}{2.303\,T} + C' \tag{5}$$

By substitution of the value of 4963 for ΔH, and taking the values of K from Tables I–IV, we may obtain values for C' the mean of which is 3.3652. We may thus calculate the value of the equilibrium constant at any temperature by the equation,

$$\log K = \frac{-1084}{T} + 3.3652 \tag{6}$$

In Table V is given a comparison of the experimental results with those calculated from this equation.

Table V

Comparison of Calculated and Observed Results

Temp., °C.	25	50	75	100
K, calcd.	0.541	1.024	1.604	2.830
K, obs.	0.540	1.022	1.593	2.890

It is apparent that the agreement is extremely good.

II. The Effect of Hydrogen Ion

Introduction.—Examination of the literature develops the facts (a) that in solutions containing an excess of strong mineral acid creatine may be practically quantitatively converted into creatinine at somewhat elevated temperatures; (b) that creatinine may be at least partially converted into creatine in alkaline solutions. There is considerable divergence of opinion as to the best conditions for effecting these transformations, and concerning the equilibrium conditions at intermediate hydrogen-ion concentrations no data at all are available. The present paper contains an experimental and theoretical study of the effect of hydrogen ion upon the creatine-creatinine equilibrium.

Experimental Part

Materials.—The creatine and creatinine used were the same as those employed for the work on aqueous solutions. Solutions of known hydrogen-ion concentration were prepared from materials of highest purity in accordance with the procedure recommended by Clark.[8] The solutions employed were phthalate-hydrochloric acid, phthalate-sodium hydroxide, acetate-acetic acid, glycine-hydrochloric acid, and potassium chloride-hydrochloric acid mixtures.

Analytical.—The analytical methods for creatinine were the same as those previously employed.

Apparatus and General Technique.—The method employed in determining the creatine-creatinine equilibrium at various hydrogen-ion concentrations was similar to that employed with aqueous solutions, except that the creatine-creatinine mixtures were dissolved in buffer solutions of known hydrogen-ion concentrations. The buffer solutions were made up to twice the desired concentration and were then diluted with an equal volume of a solution containing creatine and creatinine in various ratios, the combined concentration being always 0.001 *M*. The mixtures were heated for various lengths of time and were then analyzed for creatinine. Preliminary determinations were carried out as before to ascertain the approximate equilibrium point, and final determinations were then made to fix the point more exactly. The hydrogen-ion concentrations of most solutions were checked, after mixing, by the electrometric method, and

TABLE VI

RESULTS WITH VARIOUS BUFFER SOLUTIONS

BUFFER SOLUTION: PHTHALATE-SODIUM HYDROXIDE

PH	Mole per cent. added Creatine	Mole per cent. added Creatinine	Mole per cent. found Creatinine	Creatinine/creatine (m/n)
6.2	47.5	52.5	52.62	
	47.0	53.0	52.49	1.11
	46.5	53.5		
5.8	40.0	60.0		
	45.0	55.0	55.24	1.23
	50.0	50.0	55.39	
5.2	40.0	60.0	57.03	
	42.0	58.0	57.13	1.32
	44.0	56.0	57.17	
4.6	41.0	59.0	59.92	
	40.0	60.0	60.04	1.50
	39.0	61.0	60.11	
4.2	39.0	61.0	61.56	
	38.0	62.0	61.44	1.62
	37.0	63.0		

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[8] Clark, "The Determination of Hydrogen Ions," Williams and Wilkins Co., Baltimore, 1923.

TABLE VI (*Concluded*)

pH	Mole per cent. added Creatine	Creatinine	Mole per cent. found Creatinine	Creatinine/creatine (m/n)
BUFFER SOLUTION: PHTHALATE-HYDROCHLORIC ACID				
3.8	38.0	62.0	64.79	
	36.0	64.0	64.64	1.84
	34.0	66.0	64.84	
3.6	30.0	70.0	66.69	
	32.0	68.0		2.04
	34.0	66.0	66.51	
3.2	23.0	77.0	75.21	
	25.0	75.0	74.84	2.98
	27.0	73.0	75.12	
BUFFER SOLUTION: ACETATE-ACETIC ACID				
5.91	40.0	60.0	55.52	
	45.0	55.0		1.24
	50.0	50.0	55.24	
5.29	40.0	60.0	56.96	
	42.0	58.0		1.33
	44.0	56.0	56.98	
3.92	40.0	60.0	63.38	
	45.0	55.0	63.28	1.72
	50.0	50.0	63.19	
3.14	42.0	58.0		
	43.0	57.0	75.50	
	44.0	56.0	74.80	
	23.0	77.0	75.49	2.97
	24.6	75.4	75.49	
	27.0	73.0	75.34	
BUFFER SOLUTION: GLYCINE-HYDROCHLORIC ACID				
2.88	19.0	81.0	82.80	
	20.0	80.0	82.30	
	21.0	79.0	82.52	4.61
	16.0	84.0		
	17.6	82.4	81.64	
	20.0	80.0	81.90	
2.61	8.0	92.0	89.10	
	10.0	90.0	88.94	8.17
	12.0	88.0	89.26	
1.93	3.0	97.0		
	4.0	96.0	97.04	
	5.0	95.0	97.04	32.13
	2.0	98.0	96.97	
	3.0	97.0	96.99	
	4.0	96.0	96.90	
BUFFER SOLUTION: HYDROCHLORIC ACID-POTASSIUM CHLORIDE				
1.80	2.0	98.0	97.09	33.36
2.20	7.0	93.0	92.59	12.51

it was found that the values agreed very closely with those calculated, showing that the addition of the very small amount of creatine and creatinine did not measurably affect the hydrogen-ion concentration. The determinations were all made at 50°, a temperature at which equilibrium is reached rather rapidly, and at which complete data for solutions in pure water are available. It is expected that the experiments will later be extended to other temperatures.

Experimental Data

The data obtained using the five buffer solutions are shown in Table VI. From the equilibrium mixtures the ratios

$$\frac{m}{n} = \frac{\text{Concentration of creatinine (total)}}{\text{Concentration of creatine (total)}}$$

have been calculated and are shown in the last column of each table for each unit of hydrogen-ion concentration. The mixtures were heated at 50° for 75 hours and the total concentrations were 0.001 *M* in every case.

Discussion of Data

It is clear from the data (a) that the effect of changing hydrogen-ion concentration on the creatine-creatinine equilibrium is very marked, the equilibrium shifting towards the creatinine side with decreasing P_H; (b) the nature of the buffer solution is unimportant; (c) the effect is particularly marked between P_H 1.5 and P_H 3.0; (d) the *rate* at which equilibrium is reached increases with increasing hydrogen-ion concentration.

Theoretical Considerations

(a) Creatine and creatinine are both weak bases, creatinine being considerably more highly ionized (see below), and it would appear that the effect of hydrogen-ion concentration upon the equilibrium between the two substances should be capable of theoretical treatment. The relative proportions of molecular and ionized creatine and creatinine may be expected to change rapidly with changing hydrogen-ion concentration, while analytical methods do not distinguish between them. A mathematical analysis of the situation does much to explain the experimental data.

(b) **Notation**: m is the concentration of creatinine as experimentally measured, that is, total creatinine in all forms (moles per liter); n is the concentration of creatine (total of all forms); C_1, the concentration of creatinine as such; C_2, the concentration of creatine as such; $C_1{}^+$, the concentration of creatinine ion; $C_2{}^+$, the concentration of creatine ion; C_{OH^-}, the concentration of hydroxyl ion; K_1, the basic ionization constant of creatinine; K_2, the basic ionization constant of creatine; K_3, the equilibrium constant for molecular creatine and creatinine (see Part **I**).

Assumptions.—(a) Creatinine and creatine are weakly ionized as bases in aqueous solution according to the equations

$$\text{Cr}_1(\text{H}_2\text{O}) = \text{Cr}_1{}^+ + \text{OH}^- \qquad (7)$$

and

$$Cr_2(H_2O) = Cr_2^+ + OH^- \qquad (8)$$

Any ionization of these substances as acids is negligible.

(b) Salts of creatinine and creatine in dilute aqueous solutions are entirely ionized, or rather, the concentration of such substances as Cr_1HCl is negligible.

(c) The activities of all substances are approximately proportional to their concentrations in dilute solution.

Fundamental Equations.—From Equations 1 and 2

$$\frac{C_1^+ \times C_{OH^-}}{C_1} = K_1 \qquad (9)$$

$$\frac{C_2^+ \times C_{OH^-}}{C_2} = K_2 \qquad (10)$$

$$\frac{C_1}{C_2} = K_3 \qquad (11)$$

From Assumptions a and b

$$m = C_1 + C_1^+ \qquad (12)$$

$$n = C_2 + C_2^+ \qquad (13)$$

By a series of simple mathematical transformations we arrive at the equation

$$\frac{m}{n} = \frac{K_3(C_{OH^-} + K_1)}{(C_{OH^-} + K_2)} \qquad (14)$$

or

$$\frac{m}{n} = \frac{K_3\left(\frac{K_w}{10^{-P_H}} + K_1\right)}{\frac{K_w}{10^{-P_H}} + K_2} \qquad (15)$$

Certain qualitative conclusions can be drawn at once from Equation 14 as to the effect of hydrogen ion upon the ratio m/n. When K_1 and K_2 are small compared with C_{OH^-}, Equation 14 becomes simply

$$\frac{m}{n} = K_3 \qquad (16)$$

When K_1 and K_2 are large compared with C_{OH^-}, Equation 14 becomes

$$\frac{m}{n} = \frac{K_3K_1}{K_2} \qquad (17)$$

These two equations represent the limiting equilibrium conditions in (say) alkaline and strongly acid solutions, respectively. For intermediate values, quantitative values for K_1 and K_2 are necessary. Unfortunately, as in the case of most weak bases, there is considerable uncertainty in these values. Three values for K_1 occurring in the literature vary a hundred fold. The best data for both constants are apparently those of Hahn and Barkan[3] who give at 17°, $K_1 = 1.85 \times 10^{-10}$ and $K_2 = 4 \times 10^{-12}$. In the absence of any data whatever on the temperature coefficients of the ionization constants we may as a first approximation use these values at any temperature. Considering now Equation 14, it is clear that for pure aqueous solutions C_{OH^-} is always very large compared with K_1 and K_2, and therefore the ratio m/n gives correctly the true value for the equilibrium constant for the reaction between *molecular* creatine and creatinine. Furthermore, any increase in C_{OH^-}, making the solution alkaline, will not affect m/n, so that the same values for the equilibrium constant will be obtained in alkaline as in neutral solutions.

On the other hand, in solutions, say, 0.5 M in hydrochloric acid, C_{OH^-} is negligible with respect to K_1 and K_2 and m/n reaches another limiting value, K_3K_1/K_2. If K_1/K_2 has the value of 46 (see above) it follows that nearly all of the creatine will have been converted into creatinine in such a solution, the fraction depending upon the value of K_3 which in turn depends upon the temperature (see Part I).

When we compare Equation 14 with the results of the experimental data it is clear that a series of theoretical values for m/n can be calculated if we assume that K_1 and K_2 have the values given above, and take the ionization constant for water from the literature.[9] For example, at 50°, if $P_H = 2$, $C_H = 0.01$ and $C_{OH^-} = 5.66 \times 10^{-12}$. Hence $m/n = \dfrac{1.02(5.66 \times 10^{-12} + 1.85 \times 10^{-10})}{5.66 \times 10^{-12} \times 4.6 + 10^{-12}} = 18.98$. Similar calculations at other values for P_H give, at $P_H = 1$, $m/n = 36.72$; $P_H = 3$, $m/n = 4.01$; $P_H = 4$, $m/n = 1.33$; $P_H = 5$, $m/n = 1.05$; $P_H = 6$, $m/n = 1.03$.

In Fig. 1 have been plotted the theoretical values for m/n at various values for P_H, as outlined above, and also the experimental values. It is obvious that the agreement is excellent, being really better than would be expected considering the uncertainty in the values of K_1 and K_2. Similar types of curve must be obtained at other temperatures. Even if the values employed for K_1 and K_2 are in error, consideration of Equation 14 shows that the same *type* of curve as that shown in Fig. 1 must always be obtained.

Summary

1. The equilibrium between creatine and creatinine has been studied in aqueous solution at various temperatures.

2. An equation has been derived for the effect of temperature upon the equilibrium.

3. The effect of hydrogen ion upon the equilibrium has been studied experimentally.

4. A theoretical discussion has been given of the effect of hydrogen ion, and an equation has been derived for the effect of hydrogen ion upon the equilibrium at any given temperature.

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[9] Lewis and Randall, "Thermodynamics and Chemistry," McGraw-Hill Book Co., **1923**.