CXV. THE CREATINE-CREATININE EQUILIBRIUM. THE APPARENT DISSOCIATION CONSTANTS OF CREATINE AND CREATININE.

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The facile conversion of creatine into creatinine under the influence of strong acids and the partial reversal of the reaction in neutral and in alkaline solutions have long been familiar. Yet only recently has there become available any quantitative data upon the equilibrium conditions. Hahn and Barkan [1920] were the first to report any systematic kinetic studies. They determined the equilibrium constant in solutions of sodium hydroxide of varying concentration, observed that the component velocities increased with increasing $[OH^-]$ and showed that the order of the reaction creatinine→creatine, under these conditions, was that of a reversible monomolecular system. In a molar solution of hydrochloric acid, on the other hand, the reverse reaction went to completion and followed the course of a simple monomolecular change. Hahn and Meyer [1923] later reported a few observations which indicated that the velocity of this reaction in buffered solutions increased rapidly from p_H 6 to 4. A more elaborate study of this system has been made by Edgar and his associates. Edgar and Wakefield [1923] determined the monomolecular velocity constants (k_2) of the dehydration of creatine in hydrochloric acid solutions of varying concentration and at various temperatures. They succeeded in relating k_2 to the temperature by means of the Arrhenius equation and, further, concluded that k_2 was, probably, proportional to the hydrogen ion activity. Finally, Edgar and Shiver [1925] have made an extensive series of determinations of the equilibrium constant (K) at 50° in buffered solutions of p_H values 1 to 6. Hahn and Barkan had suggested that their observations could be interpreted upon the assumption that the molecular species whose concentrations determined the equilibrium were the undissociated molecules of creatine and of creatinine. Confirming this, Edgar and Shiver obtained fairly satisfactory agreement between the observed values of K and those calculated from the dissociation constants of the two bases and the value of K in unbuffered solution (*i.e.* where the two reactants were not significantly dissociated).

Consideration of the results summarised above will indicate that, although the hypothesis of Hahn and Barkan has been useful in co-ordinating the equilibrium data, it fails to comprehend the relations between $[H^+]$ and the velocities of the reactions. If the equilibrium be determined by the ratio of the concentrations of the undissociated molecules of creatine and creatinine, the velocities of the two contributing reactions should be governed by the same factors. Thus, if k_b be the dissociation constant of either reactant and k the velocity constant for its decomposition into the other, then k should vary with $\frac{[OH^-]}{k_b+[OH^-]}$. That is to say, the velocity should be inversely proportional to $[H^+]$ on the acid side of the buffer range of k_b and should be independent of $[H^+]$ on the alkaline side. The available experimental evidence, however, indicates that the velocities are proportional to $[H^+]$ in solutions of strong acids and are inversely proportional in strongly alkaline solution. The reactions under discussion are of such direct biological interest that it was decided to undertake a series of kinetic studies in buffered solutions between p_H 1 and 10 as an attempt to elucidate these discrepancies.

It was necessary, in the first place, that there should be available dependable values for the dissociation constants of creatine and creatinine. Since the values in the literature differ rather seriously, a redetermination of these constants was undertaken.

DISSOCIATION CONSTANTS OF CREATINE AND CREATININE.

The method employed was that of electrometric titration of dilute solutions of the two bases with standard hydrochloric acid in the presence of the hydrogen electrode. The routine technique of this laboratory has already been described [Cannan and Knight, 1927]. The reference electrode was a saturated calomel cell which was standardised against 0·05 *M* acid potassium phthalate [Clark, 1922]. Two palladinised gold-plated platinum electrodes were employed as duplicate hydrogen electrodes. No difficulty was encountered in attaining stable potentials in any of the solutions titrated and the two electrodes agreed within 0·3 mv. at all significant points on the titration curves.

The creatine was prepared from a good commercial sample by repeated recrystallisation from water. After drying to constant weight over calcium chloride, a typical preparation gave

Nitrogen (Kjeldahl) ...	28·19 %	Water 12·18 %
Theory for $C_4H_9O_2N_3.H_2O$	28·19	12·08

A saturated solution gave no reaction for creatinine upon applying Weyl's test.

Creatinine was prepared from the creatine by treating the latter with hydrochloric acid gas and subsequent liberation of the base by aqueous ammonia. The product was recrystallised from acetone [Edgar and Hinegardner, 1923]. Nitrogen and water determinations were quantitative for anhydrous creatinine. Folin's colorimetric method for the determination of creatinine (using creatinine picrate as standard) gave results in agreement with the nitrogen values, but this method is, admittedly, not sufficiently accurate to detect traces of impurity in creatinine.

It will be convenient, throughout the paper, to conduct the discussion in terms of hydrogen ions rather than of hydroxyl ions and, consequently, all constants will be treated as though they were acid constants. That is to say, the kation of a base will be regarded as an acid which dissociates a hydrogen ion [Bronsted, 1923]. The constants so derived (k') are related to the familiar k_b values by the equation $p_{k'} = p_{k_w} - p_{k_b}$.

Table I. *Uncorrected apparent dissociation constant of creatinine.*

Authors	Molar conc.	Temp.	$p_{k'}$	k_{υ}
Wood [1903]	0·1	40·2	2·97	$3{\cdot}57 \times 10^{-11}$
McNally [1926]	—	40·0	4·42	$1{\cdot}01 \times 10^{-9}$
Cannan and Shore	0·1	30·0	4·77	$0{\cdot}98 \times 10^{-9}$
,,	0·02	30·0	4·72	$0{\cdot}15 \times 10^{-9}$
,,	0·1	25·0	4·78	$0{\cdot}76 \times 10^{-9}$
McNally [1926]	—	25·0	4·71	$0{\cdot}70 \times 10^{-9}$
Eadie and Hunter [1926]	0·1	20·0	4·87	$0{\cdot}64 \times 10^{-9}$
Hahn and Barkan [1920]	0·04	17·0	4·44	$0{\cdot}19 \times 10^{-9}$
Cannan and Shore	0·02	15·0	4·91	$0{\cdot}47 \times 10^{-9}$

Wood, and Hahn and Barkan calculated k_b from the degree of hydrolysis of solutions of the hydrochloride; Eadie and Hunter employed the electrometric titration; McNally's results are the mean of results from the conductance, hydrogen ion concentration and distribution of the hydrochloride.

In Table I are assembled several determinations of $p_{k'}$ for creatinine together with values calculated from the k_b values recorded in the literature. The important effect of temperature upon the constant is evident and renders difficult the comparison of the results of different observers. But it would seem that, apart from the two earliest determinations which were made with methods open to considerable experimental errors, the various values are in substantial agreement. It is unnecessary, therefore, to report our experimental data in any greater detail. For purposes of the analysis of the kinetic studies which follow, the value for the dissociation constant of creatinine at 30° will be taken to be $k' = 1{\cdot}90 \times 10^{-5}$, *i.e.* $p_{k'} = 4{\cdot}72$.

The case of creatine is less satisfactory. The various determinations are summarised in Table II. In Table III is given the analysis of a typical titration curve to indicate the degree of concordance of the data. The calculations have been made with the aid of the Henderson-Hasselbalch equation. The values of $[H^+]$, used in calculating the "corrected equivalents of acid," are obtained from the observed p_H after correction for the activity of the hydrogen ion by the equation $\log \tau_H = 0{\cdot}20 \sqrt{\Sigma iv^z}$ [Simms, 1926]. τ_H is the activity coefficient ratio for the hydrogen ion, Σiv^z is the sum of all the ion concentrations each multiplied by the z power of its valency. The value of z was assumed to be unity. The constants have not been corrected for activity.

Table II. *Uncorrected apparent dissociation constants of creatine.*

Authors	Molar conc.	Temp.	k'_1	$p_{k'_1}$
Wood [1903]	0·1	40·2	$2{\cdot}1 \times 10^{-3}$	2·68
Cannan and Shore	0·1	30·0	$2{\cdot}4 \times 10^{-3}$	2·62
,,	0·02	30·0	$2{\cdot}4 \times 10^{-3}$	2·62
,,	0·1	25·0	$2{\cdot}2 \times 10^{-3}$	2·66
Eadie and Hunter [1926]	0·05	20·0	$0{\cdot}9 \times 10^{-3}$	3·05
Hahn and Barkan [1920]	0·04	17·0	$1{\cdot}4 \times 10^{-3}$	2·85
Cannan and Shore	0·05	17·0	$2{\cdot}45 \times 10^{-3}$	2·61

Table III. *Titration of* 50 cc. 0·02 *M creatine with* 0·1 *M hydrochloric acid.*

Titre	p_H	$[H^+]$ corrected	Corrected equiv. acid $= \frac{[HCl]-[H^+]}{[creatine]}$	$\log \frac{a}{1-a}$	$p_{k'_1}$
0·00	5·77	—	0·00	—	—
0·20	4·41	$39{\cdot}0 \times 10^{-6}$	0·0181	−1·74	2·67
0·52	3·95	$11{\cdot}2 \times 10^{-6}$	0·0452	1·33	2·62
1·02	3·64	$23{\cdot}4 \times 10^{-6}$	0·0901	1·01	2·63
2·01	3·30	$51{\cdot}3 \times 10^{-6}$	0·1744	0·68	2·62
3·02	3·08	$85{\cdot}1 \times 10^{-6}$	0·2568	0·47	2·63
4·00	2·92	$123{\cdot}0 \times 10^{-6}$	0·3337	0·30	2·62
4·98	2·79	$166{\cdot}0 \times 10^{-6}$	0·4064	0·16	2·63
6·00	2·67	$224{\cdot}0 \times 10^{-6}$	0·4749	−0·04	2·63
8·02	2·48	$347{\cdot}0 \times 10^{-6}$	0·6010	+0·18	2·64
10·00	2·34	$501{\cdot}0 \times 10^{-6}$	0·7000	0·36	2·70
13·03	2·15	$794{\cdot}0 \times 10^{-6}$	0·8016	0·61	2·76

It is probable that the last two calculations suffer by reason of the uncertainty of the correction for hydrogen ion activity.

It will be seen from Table II that differences exist between the determinations of different observers which cannot be attributed to differences of temperature or of concentration. In particular, it is difficult to explain the conflicting results of Eadie and Hunter and of ourselves since the same method was employed and was prosecuted with the same degree of precision. No plausible source of error in the titrimetric method peculiar to creatine suggests itself. The possibility of a significant amount of conversion of creatine into creatinine during the course of a titration seems to be excluded by the velocity measurements recorded in the second part of this paper. Provisionally we will take the value for k' at 30° as $2{\cdot}40 \times 10^{-3}$, *i.e.* $p_{k'} = 2{\cdot}62$.

A question of some interest to the chemical behaviour of creatine arises from a consideration of its electrolytic dissociation. The conventional formula for creatine contains both a carboxyl and an amino-group. Creatine might be expected to behave, therefore, as an ampholyte. Only basic properties are, however, evident in its chemical behaviour and only one dissociation constant is detected by titration. This is, therefore, described as a basic constant. Hahn and Fasold [1925] have, however, found that the solubility of creatine in solutions of sodium hydroxide is greater than in water and they conclude that some dissociation of creatine as an acid occurs in solutions of great hydroxyl concentration. From their observations they calculate a value of 14·28 for p_{k_a}. Now the allocation of the first dissociation constant of creatine to the amino-group and the assignment of only negligible acid properties to the carboxyl is difficult to justify upon the grounds of organic chemical experience. Yet it is usual to describe creatine as a base. A more plausible interpretation of the acid-base behaviour of this substance would seem to follow the application to it of Bjerrum's [1923] treatment of the amino-acids. The first constant ($k_1' = k_w/k_b$) then becomes the acidic constant and the second—in this case, inaccessible—constant (k_2') is the association constant of the basic group. With this assignment of constants creatine becomes an acid comparable in strength with other carboxylic acids. At the same time a new difficulty is created for it is required that the basic dissociation shall be as great as that of the alkali hydroxides. It would be difficult to concede this to a simple amino-group and it is of interest, therefore, that creatine does not behave as a primary amine either towards nitrous acid or towards formaldehyde. In this connection the strong basic properties and anomalous behaviour of guanidine itself will be recalled. It is significant that several of the structural formulae which have been proposed to explain the anomalous reaction with nitrous acid contain a nitrogenous group which might be expected to dissociate strongly as a base [Hunter, 1928, p. 99].

The above considerations in no way prejudice the application of the dissociation constants to the co-ordination of kinetic data. It is a matter of no immediate moment whether the velocity of dehydration of creatine is determined by the concentration of undissociated creatine or of "zwitterion" —the mathematical relation to k_1' remains unmodified.

CREATINE-CREATININE EQUILIBRIUM.

Solutions of creatine (0·0106 M) and of creatinine (0·00354 M) were prepared in a series of the 0·05 M buffers recommended by Clark [1922]. The mixtures were covered with 10 cc. of toluene and stored in stoppered bottles in an air-bath maintained at 30° ± 1°. At intervals appropriate to each experiment, a sample was removed and the concentration of creatinine present was determined by the method of Folin. The standard solutions for this method were prepared from a purified specimen of creatinine picrate. The p_H values of the various reaction mixtures were determined at the beginning, and again at the conclusion, of each experiment by means of the hydrogen electrode. At the end of each experiment determination was also made of the total creatine + creatinine. In agreement with other investigators it was found that some conversion occurred of these two substances into products which reacted neither as creatine nor as creatinine. The extent of the loss during the period of experiment varied from 0·5 to 5 % according to the p_H of the solution. In view of the temperature at which the solutions were maintained and of the precarious antiseptic properties of toluene over long periods, the occurrence of bacterial decomposition may be suspected. This source of error cannot be absolutely excluded but the results are so concordant amongst themselves and fit in so well with the equilibrium data of Edgar and Shiver (obtained at temperatures from 25 to 100°) and the relation of the losses to p_{H} is such that we are persuaded that the observed destruction of reactants was not due to bacteria but to irreversible chemical decomposition to an extent similar to that recorded by the earlier observers. In any case these irreversible changes are not sufficient to explain the gross relations between the velocities and p_{H} which will be established.

Fig. 1 presents a summary of one series of observations upon the two reactions. It presents several unexpected relations of the velocities to p_{H}—notably the well-defined p_{H} optima.

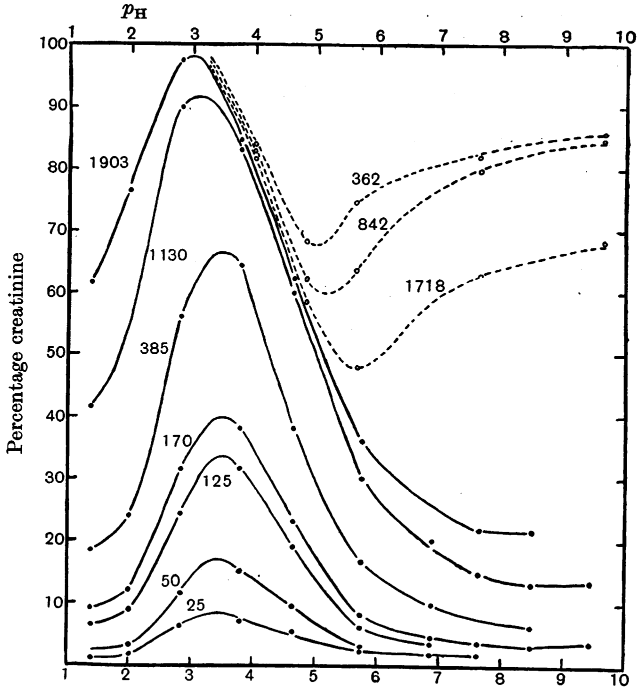

Fig. 1.

● Percentage creatinine formed in solutions of creatine at times (hours) indicated.
o Percentage creatinine remaining in solutions of creatine.

The data upon the change creatine→creatinine are more extensive than those for the reverse reaction and the analysis of the former will suffice to bring out all the important relations. The velocity constants for the latter reaction, in the p_{H} range where it is significant, fully confirm these relations.

In solutions acid to about p_{H} 3 the conversion of creatine into creatinine is seen to be substantially irreversible. In such solutions the reaction would be expected, therefore, to proceed as one of the first order. The first half of Table IV indicates the measure of constancy of the monomolecular velocity constants (k_2) derived from a typical experiment and the slight effect of allowing for the reverse reaction. The results are as satisfactory as could be expected in view of the limits of error of the colorimetric method, the slight changes in p_H accompanying the reaction and the simultaneous irreversible destruction of creatine and creatinine. In solutions alkaline to p_H 3 the system is significantly reversible. The appropriate velocity equation may be put in the form

$$k_1 + k_2 = \frac{1}{t}\, ln\, \frac{Ka}{Ka-(K+1)x} \qquad \text{......(1)},$$

where k_1 is the monomolecular velocity constant for the hydration of creatinine, k_2 is the monomolecular velocity constant for the dehydration of creatine, $K = k_2/k_1$, while a and x have their usual significance.

If K be known, this equation may be solved for k_1 and k_2. Edgar and Shiver give the following relation for K (when the reactants are not measurably dissociated, *i.e.* in unbuffered solution)

$$\log K = \frac{-1084}{T} + 3{\cdot}3652.$$

When this is solved for a temperature of 30° the value of K is given as 0·6125. According to the same authors K is related to [H+] by an equation which (when k' values are substituted for k_b values) takes the form

$$K = 0{\cdot}6125\, \frac{k''[k'+[H^+]]}{k'[k''+[H^+]]} \qquad \text{......(2)},$$

where k' is the dissociation constant of creatinine and $= 1{\cdot}90 \times 10^{-5}$, and k'' is the dissociation constant of creatine $= 2{\cdot}40 \times 10^{-3}$. From equation (2) we have calculated the values of K at the various p_H values of our reaction mixtures. When these are inserted in equation (1) together with the corresponding velocity data, the term $k_1 + k_2$ is found to be reasonably constant within a single velocity experiment. One such result is summarised in the second part of Table IV, in which values of $k_1 + k_2$ are contrasted with the values of k_2 calculated as a monomolecular velocity constant. Finally, k_1 and k_2 have been calculated for each experimental p_H and the results are assembled in Table V. This Table is restricted to the same series of experiments as are shown in Fig. 1, while in Fig. 2 the values of k_1 and k_2 have been derived from two series of observations on the rate of dehydration of creatine and one series on the rate of hydration of creatinine. The curves have been further extended into the extremes of acidity and alkalinity by the rough calculation of the values of k_1 and k_2 from the observations of Edgar and Wakefield and of Hahn and Barkan respectively. These involve an uncertain temperature correction and can only be regarded as approximate.

Restricting further discussion to the range of p_H covered by our own observations the chief point of interest is that although equation (2) satisfies the equilibrium data it is not adequate to define the velocity relations. That is to say, the individual velocity constants display a relation to [H+] which is not apparent in the equilibrium constant. The particular relation is the retardation of both velocities alkaline to p_H 3. Since this is not reflected in a change in the equilibrium constants the factors responsible must have the same influence upon the two reactions. Indeed, it may be surmised that the same factor is responsible for the changes in both k_1 and k_2 within this range.

Table IV. *Rate of dehydration of creatine* (0·0106 M) 30°.

$$p_{\mathrm{H}}\ 2{\cdot}00:\ K=\frac{[\text{creatinine}]}{[\text{creatine}]}=62{\cdot}3.$$

t	a	x	$k_2=\frac{1}{t}\,ln\,\frac{a}{a-x}$	$k_1+k_2=\frac{1}{t}\,ln\,\frac{Ka}{Ka-(K+1)\,x}$
25	100	1·97	$80{\cdot}0\times10^{-5}$	$80{\cdot}0\times10^{-5}$
75		5·58	$76{\cdot}4\times10^{-5}$	$76{\cdot}4\times10^{-5}$
125		9·12	$76{\cdot}4\times10^{-5}$	$76{\cdot}4\times10^{-5}$
170		12·25	$76{\cdot}8\times10^{-5}$	$76{\cdot}8\times10^{-5}$
385		24·33	$72{\cdot}5\times10^{-5}$	$73{\cdot}1\times10^{-5}$
865		46·18	$71{\cdot}5\times10^{-5}$	$72{\cdot}9\times10^{-5}$
1346		59·25	$66{\cdot}7\times10^{-5}$	$68{\cdot}3\times10^{-5}$
2017		76·92	$72{\cdot}7\times10^{-5}$	$75{\cdot}2\times10^{-5}$
p_{H} 3·77: $K=5{\cdot}673$.				
25	100	7·29	$304{\cdot}0\times10^{-5}$	$350{\cdot}0\times10^{-5}$
50		15·39	$334{\cdot}0\times10^{-5}$	$398{\cdot}0\times10^{-5}$
75		21·77	$327{\cdot}0\times10^{-5}$	$393{\cdot}0\times10^{-5}$
125		31·93	$308{\cdot}0\times10^{-5}$	$377{\cdot}0\times10^{-5}$
170		38·17	$283{\cdot}0\times10^{-5}$	$350{\cdot}0\times10^{-5}$
385		64·62	$269{\cdot}0\times10^{-5}$	$370{\cdot}0\times10^{-5}$
695		79·98	$232{\cdot}0\times10^{-5}$	$405{\cdot}0\times10^{-5}$
1130		83·33	$159{\cdot}0\times10^{-5}$	$347{\cdot}0\times10^{-5}$
1896		85·00	$99{\cdot}0\times10^{-5}$	$370{\cdot}0\times10^{-5}$

Table V.

p_{H}	K from equations of Edgar and Shiver	$k_1+k_2=\frac{1}{t}\,ln\,\frac{Ka}{Ka-(K+1)\,x}$	k_1	k_2	k_2 calculated from equation (3)
1·37	73·1	$53{\cdot}0\times10^{-5}$	$0{\cdot}7\times10^{-5}$	$52{\cdot}3\times10^{-5}$	$19{\cdot}6\times10^{-5}$
2·00	62·3	$74{\cdot}0\times10^{-5}$	$1{\cdot}17\times10^{-5}$	$72{\cdot}8\times10^{-5}$	$71{\cdot}4\times10^{-5}$
2·83	29·8	$242{\cdot}0\times10^{-5}$	$7{\cdot}85\times10^{-5}$	$234{\cdot}0\times10^{-5}$	$22{\cdot}6\times10^{-5}$
3·77	5·673	$373{\cdot}0\times10^{-5}$	$47{\cdot}0\times10^{-5}$	$326{\cdot}0\times10^{-5}$	$311{\cdot}0\times10^{-5}$
4·64	1·335	$311{\cdot}0\times10^{-5}$	$133{\cdot}0\times10^{-5}$	$178{\cdot}0\times10^{-5}$	$207{\cdot}0\times10^{-5}$
5·63	0·086	$129{\cdot}0\times10^{-5}$	$76{\cdot}5\times10^{-5}$	$52{\cdot}5\times10^{-5}$	$55{\cdot}3\times10^{-5}$
6·86	0·614	$71{\cdot}4\times10^{-5}$	$44{\cdot}2\times10^{-5}$	$27{\cdot}2\times10^{-5}$	$18{\cdot}2\times10^{-5}$
7·62	0·613	$46{\cdot}1\times10^{-5}$	$28{\cdot}6\times10^{-5}$	$17{\cdot}5\times10^{-5}$	$15{\cdot}9\times10^{-5}$
8·49	0·613	$38{\cdot}5\times10^{-5}$	$23{\cdot}9\times10^{-5}$	$14{\cdot}6\times10^{-5}$	$15{\cdot}5\times10^{-5}$
9·54	0·613	$41{\cdot}5\times10^{-5}$	$25{\cdot}7\times10^{-5}$	$15{\cdot}8\times10^{-5}$	$15{\cdot}5\times10^{-5}$

It has been possible to evolve equations based on equation (2) but involving three empirical constants which define with a fair degree of accuracy the relations of k_1 and k_2 to p_{H} within the range 2 to 10. They do not cover the changes in k_1 and k_2 in strongly acid and alkaline solutions.

The equations are

$$k_1=\frac{A'k'\,[C'+[\mathrm{H}^+]]}{[k'+[\mathrm{H}^+]]\,[C+[\mathrm{H}^+]]},\quad k_2=\frac{A''k''\,[C'+[\mathrm{H}^+]]}{[k''+[\mathrm{H}^+]]\,[C+[\mathrm{H}^+]]}\qquad\ldots\ldots(3),$$

where $A'=3{\cdot}68\times10^{-3}$, $C'=0{\cdot}8\times10^{-6}$ and $C=1{\cdot}9\times10^{-5}$, $A''=2{\cdot}25\times10^{-3}$.

It will be observed that, since $K=k_2/k_1$, K becomes

$$0{\cdot}6125\ \frac{k''\,[k'+[\mathrm{H}^+]]}{k'\,[k''+[\mathrm{H}^+]]}.$$

This is identical with equation (2).

Equations of the sort developed above have little merit other than the approximate summary of a mass of data. In particular, one must be very cautious in assigning any material significance to the various empirical constants. One point cannot, however, be overlooked. The value of C is identical with the dissociation constant of creatinine. It is difficult to see in what way the dissociation of creatinine can affect the intrinsic velocity of dehydration of creatine. A possible explanation might follow the assumption that there was involved in the reactions a tautomer having a constant similar to creatinine. C' might then be regarded as a second constant of this structure or as indicating the participation of yet another intermediary. A' and A'' are merely the values which k_1 and k_2 would have were they determined only by the concentration of undissociated molecules of creatine and of creatinine respectively.

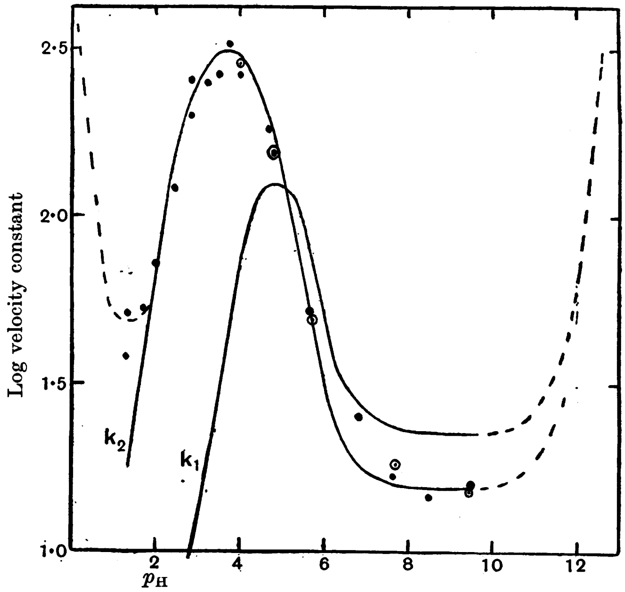

Fig. 2. Relation of velocity constants to p_H.

● Observed k_2 from rate of dehydration of creatine.

⊙ Observed k_2 from rate of hydration of creatinine.

Observed values of k_1 are not inserted as they depart from their curve to the same extent as the corresponding values of k_2.

One final point of biological interest may be mentioned. Hahn and Meyer [1923] found that at 38° in a solution of 0·5 % creatine of p_H 7·01 there appeared an amount of creatinine corresponding to 1·32 % of the total creatine in 24 hours. They calculate that the daily excretion of creatinine in the urine of an adult man corresponds to 1·33 % of the total creatine of his body. They suggest, therefore, that it is unnecessary to seek beyond the spontaneous dehydration of creatine for the origin of the creatinine of the urine. It follows from this view that the output of creatinine in the urine is governed only by the active mass of creatine in the muscles, by the temperature and by the p_H of the muscle. Hahn and his associates have made an attractive case for this simple hypothesis. The data of the present paper give a general confirmation of the above calculation. The velocity constant of the dehydration of creatine at p_H 7·2 and 30° is 23×10^{-5}. Applying the temperature correction of Edgar and Wakefield we arrive at a value of 43×10^{-5} at 38°. This corresponds to the dehydration of 1·03 % of the active mass of creatine in 24 hours. This figure—somewhat below that of Hahn and Meyer—is sufficient to account for the daily output of creatinine provided the active mass of creatine in living muscle is as great as 0·5 %. Evidence continues to accumulate, however, that this is an exaggerated value. It seems probable that only a small proportion of the total creatine which can be extracted from muscle by chemical means is in the free state in the living tissue. Unless the improbable assumption be made that combined creatine suffers dehydration as readily as when in the free state the argument of Hahn and Meyer cannot be sustained. It could then only be upheld were the demonstration made that the factors which have been shown to retard the velocity on the alkaline side of p_H 3 were partially suppressed in living muscle. It would be necessary for the apparent constant C to be diminished or the constant C' to be increased to an extent corresponding to the ratio of free creatine to total creatine in muscle. Upon this possibility there is no evidence.

SUMMARY.

1. Determination has been made of the apparent dissociation constant (uncorrected for activity) of creatinine at 15°, 25° and 30° and of the first dissociation constant (uncorrected) of creatine at 17°, 25° and 30°.

2. The velocity constants of the reversible system creatine-creatinine have been determined at 30° over the p_H range 2 to 10, and have been related to $[H^+]$, the dissociation constants of the reactants and certain empirical constants.

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