of ethylene. The more nearly normal character of the reaction at the higher temperatures is believed to be due to the fact that under these circumstances the reacting gases are not measurably adsorbed by the catalyst. The temperature coefficient is much smaller at the higher temperature and is decreasing. By taking into account the decrease of adsorption with rise in temperature as well as the normal increase in velocity of the surface reaction, these facts have been accounted for qualitatively.

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

THE KINETICS OF THE CONVERSION OF CREATINE INTO CREATININE IN HYDROCHLORIC ACID SOLUTIONS

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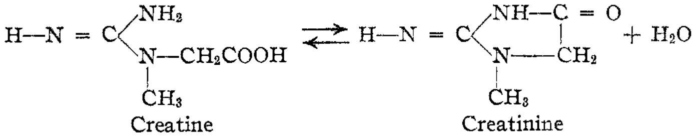
Introduction

The literature contains numerous references to the conversion of creatine into creatinine in the presence of acid catalysts. Practically all of these investigations have either been purely qualitative in nature, or have been concerned with determining the conditions under which the reaction goes to completion, in order to permit the quantitative estimation of creatine (as creatinine) by the colorimetric method of Folin.[1]

The reversible reaction



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

is of great interest in biochemistry, as it occurs in the normal and abnormal processes of the metabolism; it is also of interest from the theoretical standpoint, as it represents a type of reaction catalyzed by acids to which less attention has been paid than those of ester hydrolysis and similar reactions. The present paper represents a brief study of the kinetics of the reaction in the presence of hydrochloric acid as a catalyst.

Experimental Methods

Materials.—Commercial creatine of very good quality was purified by two crystallizations from water, and was allowed to dry in the air. The product was pure white, odorless, and finely crystalline. Other reagents used were commercial C. P. products.

As analytical standards pure creatinine picrate[2] and creatinine zinc chloride were employed.

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[1] Folin, *Am. J. Physiol.*, **13,** 48 (1905).

[2] Edgar, *J. Biol. Chem.*, **56,** 1 (1923).

Apparatus.—The reaction-velocity experiments were carried out in test-tubes fitted with rubber stoppers. These were kept at constant temperature by immersion in boiling water, alcohol or acetone, the mean temperatures being taken as 100°, 78° and 57°, respectively; a temperature of 25° was obtained by use of an electrically controlled thermostat.

For the analysis (colorimetric) a Duboscq type of colorimeter was employed, illuminated by a special colorimeter lamp.

Experimental Technique.—The pure creatine was dissolved in water and a measured quantity of the solution was put into a test-tube and brought to the desired temperature. A definite quantity of standard hydrochloric acid (previously heated to the same temperature as the creatine) was then added, and the mixture placed in the constant temperature bath. A series of similar tubes was used in each run, in order to avoid loss of time in removing and measuring samples for analysis. At the end of given time intervals, separate tubes were removed from the bath and the reaction was stopped by adding immediately a quantity of sodium hydroxide solution equivalent to the acid, and cooling rapidly. The solution was then analyzed for creatinine formed, by the well-known Folin[1] method, making use of the red color developed when creatinine is treated with picric acid and sodium hydroxide. A standard solution of creatinine (as picrate or as creatinine zinc chloride) was treated with picric acid and alkali in exactly the same way as the unknown solution, and the color was compared as usual. Repeated colorimeter readings were taken and all precautions necessary to obtain precise results with this method were observed. The accuracy is believed to be about 1% of the total creatinine present.

Results.—The results of a characteristic experiment are given in Table I, together with reaction-velocity constants calculated for a first order reaction. Table II gives the average values of the constants calculated from experiments under other conditions; the agreement of individual constants was in all cases similar to those in Table I. Table II also contains values calculated from an equation derived below.

TABLE I

RESULTS OF TYPICAL EXPERIMENT

Temperature, 78° HCl, 0.76 *M*

Time, min.	0	15	30	45	75	120	
Concn. creatine, moles/l.	0.03000	0.02500	0.02085	0.01710	0.01140	0.00660	
Vel. const., $K = \frac{1}{t} ln \frac{a}{a-x}$		0.01218	0.01200	0.01245	0.01270	0.01245	Av. 0.01235

TABLE II

AVERAGE VELOCITY CONSTANTS

Expt.	Temp. °C.	Concn. HCl Moles/liter	Initial concn. creatine Moles/liter	*K* found	*K* calc.
1	25	0.38	0.00750	0.0000340	0.0000314
2	57	.38	.0300	.000777	.000824
3	78	.19	.00447	.00256	.00254
4	100	.38	.0075	.02816	.0278
5	78	.38	.0075	.00494	.00512
6	100	.19	.00367	.01367	.0138
7	78	.76	.0300	.01235	.01245
8	78	.76	.0150	.01256	.01245

Discussion

General.—Reaction 1 proceeds in pure water to a condition of equilibrium[3] in which the ratio of creatine to creatinine is of the order of unity. Creatinine is, however, much more basic than creatine, and in the presence of acid the reaction goes "to completion" with the formation of a salt of creatinine.[4] It is obvious from the data in Table I, (confirmed by the actual data from which Table II is constructed) that under these conditions of experiment the reaction is essentially unimolecular. Whether or not an actual bimolecular reaction with the hydrogen-ion catalyst is involved[5] cannot be determined from the data, although it seems probable from the data given in the following paragraph. It may be noted that combination of the creatinine with the acid presumably reduces the concentration of catalyst somewhat as the reaction proceeds; it is therefore necessary to keep the concentration of creatine small as compared with that of the acid; this has been done in all experiments, as is shown by the data in Table II.

The Effect of Acid Concentration.—The velocity constants obviously increase with the concentration of acid, increasing from the values in 0.19 *N* hydrochloric acid to those in 0.76 *N* acid (Expts. 3, 5 and 7, Table II), somewhat more rapidly than the increase of the acid concentration, although the slope of the velocity-constant—acid–concentration curve passes through an apparent minimum around 0.4 *N* hydrochloric acid. This is in accordance with data of other investigators on other reactions catalyzed by hydrochloric acid, and it seems probable that the velocity constants are proportional to the hydrogen-ion activity, although the data do not enable an exact comparison to be made.

The Temperature Coefficient.—The data in Table II show that at any given concentration of hydrochloric acid the effect of temperature may be expressed by the Arrhenius[6] equation

$$\frac{d\,ln\,K}{dT} = \frac{E}{RT^2} \tag{2}$$

where E is the "critical increment" or "heat of activation." For a concentration of hydrochloric acid equal to 0.38 *N* the values calculated for the temperature intervals 25–57°; 57–78°; 78–100° are 19050, 20400, 20500 calories, respectively, indicating that for this temperature range E may be regarded as practically independent of the temperature. A similar calculation of the value of E for an acid concentration of 0.19 *N* and a temperature range of 78–100° gives a value of 19790 calories, indicating that for this range of concentrations E is independent of the acid con-

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

[4] Hahn and Barkan, *Z. Biol.*, **72**, 305 (1920), and many earlier references to various authors.

[5] See Garrett and Lewis, THIS JOURNAL, **45**, 1901 (1923).

[6] Arrhenius, *Z. physik. Chem.*, **4** (1889).

centration. We can therefore express the velocity constants under the whole range of conditions approximately by the integrated equation

$$ln\,K = -\frac{E}{RT} + C \qquad (3)$$

where E has the mean value of 20,000 calories, and the value of the constant C depends upon the concentration of acid. Changing to ordinary logarithms, Equation 3 becomes

$$\log K = -\frac{4368}{T} + C' \qquad (4)$$

the average values for the constants for the three acid concentrations being as follows: 0.19 N HCl, $C' = 9.8496$; 0.38 N HCl, $C' = 10.1538$; 0.76 N HCl, $C' = 10.5400$. If these values are substituted in Equation 4 and the values of K calculated for the different experiments we obtain the results given in the last column of Table II, which agree reasonably well with the experimental values. If the values of C are plotted against the acid concentration we may obtain by interpolation, values of the constant for any acid concentration within the range studied, which will enable the calculation of an approximate velocity constant at any temperature. This may be of use in determining the time necessary for practically complete conversion under various conditions, as in the analysis of creatine solutions.

It may be noted that the value for the critical increment is of the same order as that observed by various investigators for many other cases of acid catalysis in different types of reaction.

Summary

1. Measurements have been made of the rate of conversion of creatine into creatinine in hydrochloric acid solution at various temperatures and concentrations of acid.

2. The general nature of the reaction, and the influence of temperature and acid concentration are discussed.

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