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THE CONVERSION OF CREATIN TO CREATININ IN HYDROCHLORIC ACID SOLUTIONS.

BY

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I. INTRODUCTION

The literature contains numerous references to the qualitative conversion of creatin to creatinin in the presence of acid catalysts. There seems to have been a lack of any quantitative investigations of this dehydration reaction. The above mentioned qualitative data is very largely concerned with the conditions necessary to give complete conversion of the creatin in meat extracts and urine. The conversions were carried out simply as a step in the quantitative analysis for creatin, because of the fact that there is no simple direct method for the determination of creatin, while the colorimetric method for creatinin is rapid and reasonably accurate. These investigators using the reaction as a means to an end, were not concerned with the mechanism of the reaction itself.

The purpose of this paper therefore is to give a quantitative study of the dehydration reaction of creatin to creatinin in the presence of hydrochloric acid as a catalyst, noting especially the effect of acid concentration and temperature upon the speed of the reaction.

II. EXPERIMENTAL

(a) MATERIALS. Creatin, on the market now in a comparitively pure form (1), was purified by two crystallizations, and dried without the application of heat. The product was colorless, pure white, and finely crystalline.

Creatinin hydrochloride, creatinin picrate, and creatinin zinc chloride prepared from the above creatin (2) were used at different times as standards.

Other reagents used were chemically pure commercial products.

(b) DESCRIPTION OF APPARATUS. The conversions were carried out in ordinary test tubes using rubber stoppers. The tubes were kept at constant temperatures of 100°, 78°, and 57° by immersion in boiling water, alcohol, and acetone

respectively contained in beakers fitted tith reflux condensers. The 25°C temperature was obtained by the use of a thermostat.

The analyses for creatinin were made by the Folin (4) colorimetric method using a Dubosq colorimeter. It was estimated that this method could be carried out within the limits of 1-2% error. A "daylight" lamp with a blue glass lens was used to give uniform lighting.

(a) GENERAL TECHNIQUE. The desired quantity of creatin solution was put into a test tube, brought to the specified temperature and then the standard acid was added. Separate tubes were used for each analysis in order to eliminate any danger of evaporation which might result from the withdrawal of samples from one tube at different intervals. Tubes were withdrawn from the constant temperature baths from time to time. The action was immediately stopped by adding a calculated quantity of standard sodium hydroxide and cooling. The solutions were analysed at once by the Folin method (4) for creatinin formed. Simultaneously with the treatment of the sample, there was carried out an exactly similiar treatment on a

standard solution of creatinin. This standard creatinin solution was placed in the second tube of the colorimeter and used in place of the standard potassium di-chromate solution originally suggested by Folin (3). It was found best to take the average reading of the colorimeter after interchanging the tubes. A characteristic set of readings follows.

TABLE 1.

Standard on right; unknown set at 70 mm on the left.

16.6 mm
16.8 mm
16.9 mm
16.7 mm

Standard on the left; unknown set at 70 mm on the right.

16.2 mm
16.3 mm
16.4 mm

Average; standard 16.025 mm; unknown 70 mm.

This pure creatinin standard was found to be much more satisfactory than the standard di-chromate whose color varies with the depth and must be corrected by use of a certain factor (8). Creatinin pic rate was also used as a very satisfactory standard (6).

In this application of the Folin method 25cc of saturated picric acid solution and 10cc of 10% sodium hydroxide were used.

The amount of creatinin present in a sample was calculated from the colorimetric readings by the following scheme:

$$\frac{\text{weight of standard creatinin} \times \text{depth of standard in mm}}{\text{depth of unknown in mm}} \qquad (1)$$

The amount of creatin converted was calculated from the creatinin present by multiplying by the chemical factor,

$$\frac{\text{creatin}}{\text{creatinin}} = 1.32 \qquad (2)$$

The reaction velocity constants were calculated by the expression,

$$K = \frac{1}{T} \ln \frac{a}{a-x} , \qquad (3)$$

for a first order reaction, where (a) equals the amount of creatin in the sample, (x) the amount of creatin converted, and (T) the time.

(d) EXPERIMENTAL DATA:

TABLE 2

0.76 N-HCl; Temperature 78°C

Time in minutes	Creatin moles per l.	% Conversion	K. (velocity constant)
0	0.03000	0.	-------
15	0.02500	16.67	0.01218
30	0.02085	30.5	0.01200
45	0.01710	43.0	0.01246
75	0.01140	62.0	0.01270
120	0.00660	78.0	0.01245
			0.01235 average

TABLE 3

0.76 N-HCl; Temperature 78°C

Time in minutes	Creatin moles per l.	% Conversion	K. (velocity constant)
0	0.01500	0.	-------
30	0.01026	31.6	0.01285
45	0.00861	42.6	0.01235
90	0.00465	69.0	0.01270
			0.01256 average

TABLE 4

0.5$ N-HCl; Temperature 78°C

Time in minutes	Creatin moles per l.	% Conversion	K. (velocity constant)
0	0.00750	0.	-------
120	0.00417	44.4	0.00486
210	0.00258	65.5	0.00502
330	0.00140	80.5	0.00495
			0.004943 average

TABLE 5

0.1$ N-HCl; Temperature 78°C

Time in minutes	Creatin moles per l.	% Conversion	K. (velocity constant)
0	0.00447	0.	-------
240	0.00243	45.5	0.00254
360	0.00181	59.5	0.00250
570	0.00100	77.6	0.00264
			0.00256 average

TABLE 6

0.36 N-HCl; Temperature 37°C

Time in minutes	Creatin moles per l.	% Conversion	K. (velocity constant)
0	0.03000	0.	-------
600	0.01863	37.9	0.000791
1200	0.01200	60.0	0.000770
1400	0.00975	67.5	0.000770
			0.000777 average

TABLE 7

0.36 N-HCl; Temperature 25°C

Time in minutes	Creatin moles per l.	% Conversion	K. (velocity constant)
0	0.00750	0.	-------
17280	0.00416	44.5	0.00003392
21630	0.00360	51.9	0.00003380
27300	0.00293	60.8	0.00003430
			0.0000340 average

TABLE 8

0.38 N-HCl; Temperature 100°C

Time in minutes	Creatin moles per l.	% Conversion	K. (velocity constant)
0	0.00750	0.	-------
30	0.00327	56.4	0.0270
60	0.00138	81.6	0.0281
93	0.00048	93.4	0.0288
			0.02816 average

TABLE 9

0.09 N-HCl; Temperature 100°C

Time in minutes	Creatin moles per l.	% Conversion	K. (velocity constant)
0	0.00367	0.	-------
30	0.00243	33.77	0.01373
70	0.00141	61.6	0.01368
105	0.00087	76.1	0.01360
			0.01367 average

III. DISCUSSION OF DATA

(a) GENERAL NATURE OF THE REACTION: A consideration of the above data will show that the reaction is monomolecular. By a comparison of tables one and two, it will be noticed that the velocity constant remains the same altho the solutions of table one contain twice as much creatin as those of table two, ie the velocity constant of the reaction is independent of the creatin concentration. The other tables confirm this statement in that the velocity constants of all the tables vary regularly with the temperature and acid concentration regardless of the amount of creatin taken.

(b) EFFECT OF ACID CONCENTRATION: The effect of acid on the velocity constant at 78°C is shown by the graph, (Figure 1). Here the constant increases a little faster than the acid concentration expressed as normality. Possibly the curve would be linear if plotted against hydrogen ion concentration. This we cannot prove readily, due to a lack of data on the concentration of the hydrogen ion at the higher temperatures. The concentration of the hydrochloric acid in all cases was in such an excess over the creatin concentration that the amount of acid removed

from action by combining with the creatinin produced, to form creatinin hydrochloride, may be neglected and the concentration of the acid may be considered constant throuout the reaction. This was shown to be true by a preliminary examination of the work done at 57°C. In this preliminary study the reaction was carried out with the acid concentration relatively small, certainly not in excess. It was found that the velocity constant decreased regularly with time. This was explained on the assumption that the concentration of hydrochloric acid was being decreased progressively due to salt formation with the creatinin. When the amount of acid was increased to a relatively large excess there was no change in the velocity constant, Table 6. That there is a uniform relationship between acid concentration and the velocity of the reaction is definitely shown. The approximate velocity constants at 78°C may be estimated for any normality of HCl from the graph (Figure 1), as shown in Table 10.

TABLE 10.

Normality of HCl	Velocity Constant
0.25	0.00328
0.50	0.00689
0.75	0.01221

(c) EFFECT OF TEMPERATURE: The effect of temperature is very marked. The relation between the velocity constant and the absolute temperature is shown by the Arrhenius (7) equation,

$$\frac{d \ln K}{dt} = \frac{E}{RT^2}; \qquad (4)$$

where E is the "critical increment" expressed in calories, and T is the absolute temperature. Using the integrated form,

$$\ln K_1 - \ln K_2 = \frac{E (T_1 - T_2)}{R T_1 T_2}, \qquad (5)$$

of the above equation, the values of E are very constant, approximating 20,000 calories, as shown in Table 11.

TABLE 11.

Critical Increments

25° --- 37°	E = 14050 cal.)	
37° --- 78°	E = 20400 cal.)	0.38 N-HCl
78° --- 100°	E = 20500 cal.)	
78° --- 100°	E = 19790 cal.)	0.19 N-HCl

By calculating the value of the critical increment at different normalities, it is shown that E is independent of acid concentration for the range studied.

These results are of the same order as those values of the critical increments of certain other monomolecular reactions studied by W. M. Lewis; for the hydrolysis of methyl acetate, 16800 calories (8); for the inversion of sucrose, 25000 calories (8).

An approximate value of the velocity constant at any temperature and at any normality may be calculated by the use of the equation

$$\ln K = \frac{-E}{RT} + C \qquad (6)$$

The integration constant in this equation is independent of temperature and dependent on acid strength. Substituting the values of the velocity constants from Tables 2 --9 in equation 6, the average values of C for each acid concentration were calculated and plotted against normalities. From the graph (Figure 2) the value of the integration constant at any desired normality may be obtained and used in equation 6 to calculate the velocity constant at the desired normality and at any temperature. These velocity constants may also be calculated by the use of equation 5. In both methods E is assumed to equal 20000 calories. Table 12 gives a comparison of values of K calculated by the two methods.

TABLE 12

Calculation of Velocity Constants at 100°C

Normality of HCl	K(by equation 6)	K(by equation5)
0.25	0.01723	0.01772
0.50	0.03762	0.03745
0.75	0.06595	0.06657

Acid Concentration--Velocity Constant

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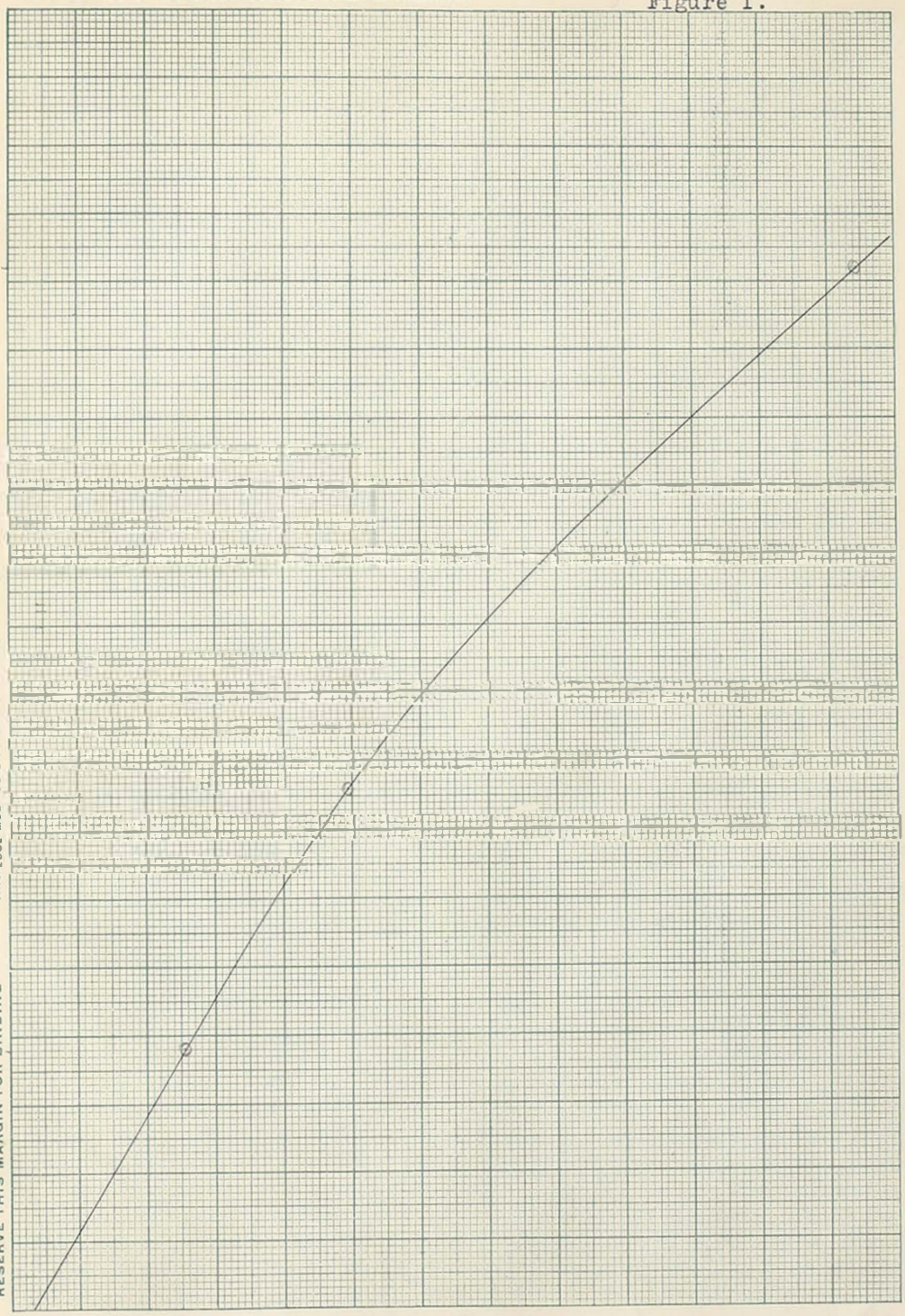
Figure I.



0.00256 0.004943 0.012435

SCALE: EACH DIVISION = 1, 0.5, 0.2, 0.1, 0.05, 0.02, 0.01, 0.005, ETC. OR = 0.5, 0.2, 0.1, 0.05, 0.02, 0.01, 0.005, ETC.

THIS EDGE MUST BE EITHER TOP OR LEFT-HAND SIDE OF SHEET

RESERVE THIS MARGIN FOR BINDING

Integration Constant--Normality of Acid

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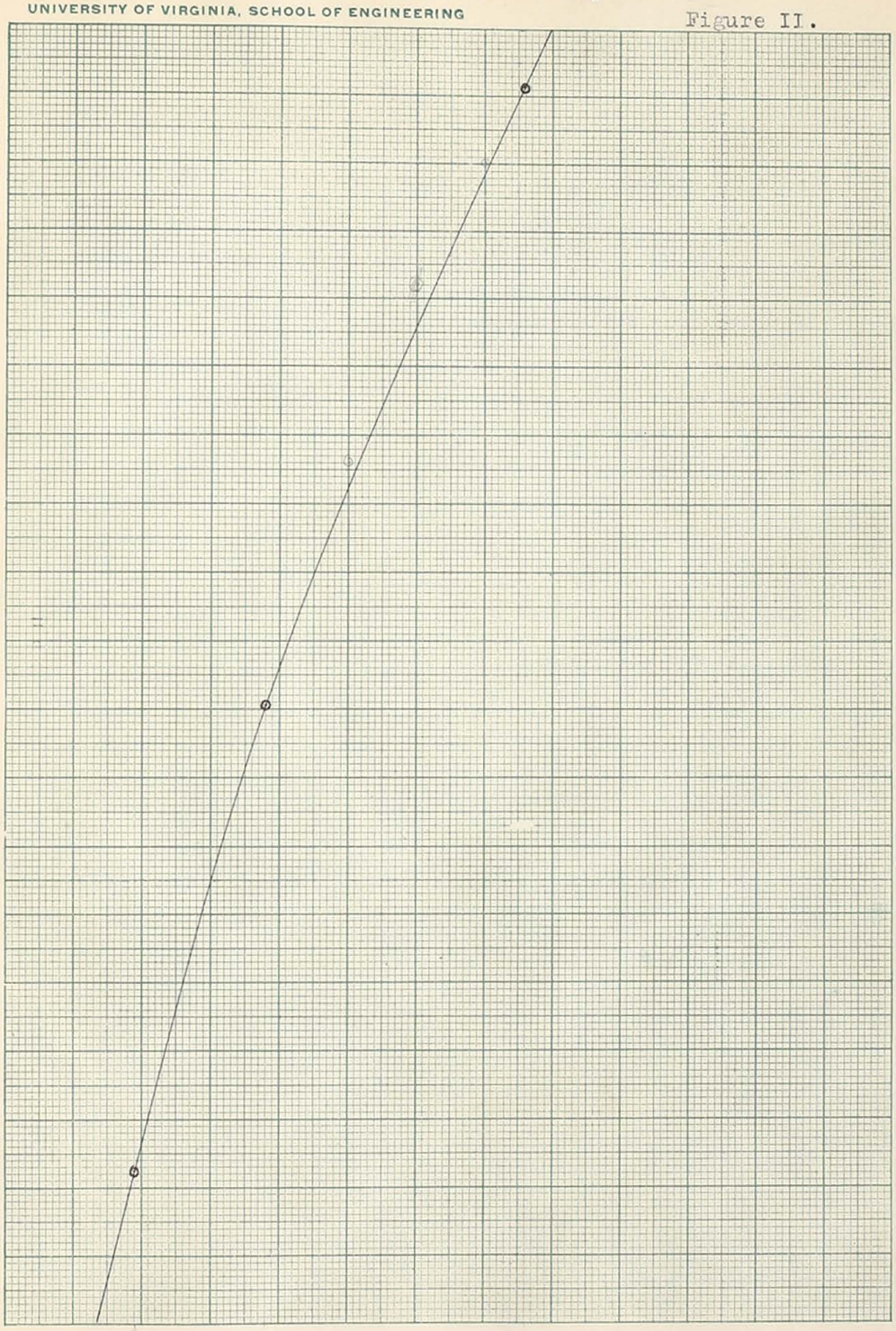
Figure II.



SCALE: EACH DIVISION — 1, 2, 5, 10, 100, 1000, ETC OR — 0.6, 0.2, 0.1, 0.01, 0.001, ETC.

THIS EDGE MUST BE EITHER TOP OR LEFT-HAND SIDE OF SHEET

RESERVE THIS MARGIN FOR BINDING

Table 13 gives a comparison of values of K as determined by experiment and as calculated by use of equation 6 assuming K equal to 20000 calories and reading the value of C from the graph, Figure 2.

TABLE 13.

Temperature	Normality of HCl	K by experiment	K by equation 6
25°C	0.38	0.0000340	0.0000309
37°C	0.38	0.000777	0.000802
78°C	0.19	0.00256	0.002523
78°C	0.38	0.004943	0.004992
100°C	0.19	0.01367	0.013716
100°C	0.38	0.02816	0.027130

One of the most valuable applications of the above data may be in the calculation of the time required for any desired percentage conversion using any normality of acid. Table 14 contains such calculations made by the use of equation 2.

TABLE 14

Time required for 99% conversion at 100°C.

Normality of HCl	Time in minutes
0.25	248.8
0.50	117.8
0.75	66.5

IV. SUMMARY

The reaction velocity constants for the conversion of creatin to creatinin by an acid catalyst have been determined.

The velocity constant of the reaction is shown to be independent of the creatin concentration. The reaction is therefore unimolecular.

The change in the rate of the reaction with different acid concentrations has been discussed.

The effect of the temperature on the reaction is demonstrated.

Values of the critical increment have been calculated. The critical increment is independent of the acid concentration for the range studied.

Various applied calculations are appended.

V. BIBLIOGRAPHY

1. Edgar, Graham, J. Ind. and Eng. Chem., 1922,14,984
2. Edgar, Grahar, J. Biol. Chem., May 1923
3. Folin O. J. Biol. Chem., 1914,17,469
4. Folin, O., Amer. J. Physiol., 1905,13,48
5. Smith and Myers, U. S. Dept. Agric. Chem., Bull.132,100
6. Thompson, J. Physiol. 1913,1
7. Arrhenius, Zeisch. Physikal. Chem.,1889,4,226
8. Lamble and Lewis, J. Chem. Soc. London, 105, 2330
9. Lamble and Lewis, J. Chem. Soc. London, 107,233