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THE RATE OF HYDROLYSIS OF SOLUTIONS OF PROTEINS IN ACIDS AS MEASURED BY THE FORMATION OF AMINO NITROGEN

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Introduction

Quantitative studies on the rates of hydrolysis of proteins in acid solution are still very few and as far as the authors are aware, no attempt has been made to determine the change of the reaction rate with change of temperature. When it is considered that in the analysis of proteins, either for all the amino acids or for groups, as in the Van Slyke scheme,[1] the proteins are first hydrolyzed in acid solution, that the usual method of doing this involves the use of a fairly strong concentration of acid (20% hydrochloric acid) and consumes a great deal of time, it seems desirable to study the hydrolysis at different temperatures and acid concentrations so as to obtain data leading to improvements in the procedure. Such studies are all the more timely now, when the many kinds of pressure cookers in common use make autoclaves cheaply available for laboratory purposes. Besides these practical considerations, quantitative studies on hydrolysis should be of aid in the solution of the problem of protein structure. The present investigation was undertaken as a step toward the ends given above. The hydrolysis of gelatin was studied at the three temperatures, 96–98°, 130° and 142°, in a number of different concentrations of hydrochloric and sulfuric acids to determine the order of the reaction, the change of reaction velocity with temperature and the effect of acid concentration on hydrolysis. Besides this, the hydrolysis of silk fibroin was also studied at 130° for the purpose of obtaining data on a protein radically different in physical properties from gelatin.

Materials and Experimental Procedure

In the experiments with gelatin, "Bacto" gelatin put up by the Digestive Ferments Company was used without any further purification. After drying in a hot-air oven at 100° it was analyzed for nitrogen and ash. The nitrogen was found to be 18.0% and the ash 1.1%.

The experimental procedure was as follows. One-g. lots of dried gelatin were put in 100cc. graduated flasks with long necks, and acid of the desired concentration was added up to the graduated mark. The autoclaving at the highest temperature (142°) was carried out in an ordinary pressure cooker, the autoclaving at 130° was carried out in a steam autoclave,

[1] Van Slyke, *J. Biol. Chem.*, **10**, 15 (1911).

TABLE I

HYDROLYSIS OF GELATIN BY HCl AT 96–98°

Time, hrs.	3.0 *N* HCl: Amino N in 100 cc. of soln., g.	3.0 *N* HCl: Hydrolysis, %	3.0 *N* HCl: *K* Second-order reaction	1.5 *N* HCl: Amino N in 100 cc. of soln., g.	1.5 *N* HCl: Hydrolysis, %	1.5 *N* HCl: *K* Second-order reaction
2	0.0826	70.3	0.0118	0.0578	49.1	0.0048
5	.0992	84.5	.0109	.0774	65.9	.0038
8	.1042	88.7	.0097	.0937	79.8	.0050
11	.1078	91.8	.0101	.1000	85.1	.0052
23	.1174	100	(−)	.1120	95.6	.0094[a]
			Av. .0106			Av. .0047
	$K/a^{\pm} = 0.0026$			$K/a^{\pm} = 0.0034$		

[a] Value not used in obtaining average.

supplied with steam at a gage pressure of 1.6 atmospheres, and the experiments at the lowest temperature were performed in an electrically-heated drying oven equipped with an automatic temperature control. In the experiments carried out in the autoclave the flasks were heated at the desired temperature for a definite length of time, after which the autoclave

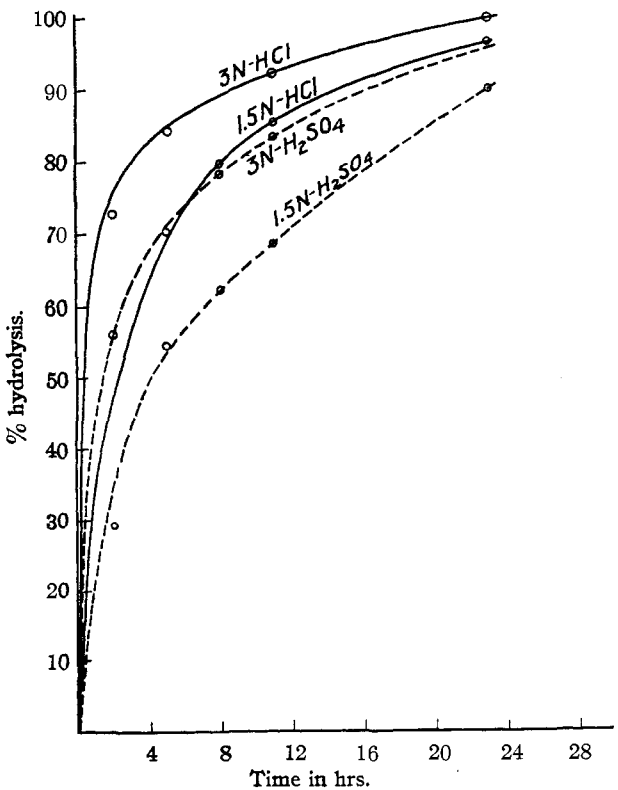

Fig. 1.—Hydrolysis of gelatin at 96–98°.

TABLE II

HYDROLYSIS OF GELATIN BY H_2SO_4 AT 96–98°

Time, hrs.	3.0 *N* H_2SO_4: Amino N in 100 cc. of soln., g.	Hydrolysis, %	*K* Second-order reaction	1.5 *N* H_2SO_4: Amino N in 100 cc. of soln., g.	Hydrolysis, %	*K* Second-order reaction
2	0.0656	55.9	0.0063	0.0345	29.4	0.0021
5	.0828	70.5	.0048	.0644	54.9	.0024
8	.0920	78.3	.0045	.0733	62.4	.0021
11	.0988	93.7	.0047	.0808	68.8	.0020
23	.1146	97.6	.0177[a]	.1043	90.3	.0040[a]
			Av. .0051			Av. .00215
	$K/4a^{\pm} = 0.0033$			$K/4a^{\pm} = 0.0028$		

[a] Value not used in obtaining average.

was cooled, the flasks were removed, cooled and the amount of liquid lost by evaporation was replaced with water. One-cc. samples were then withdrawn and placed in small tubes for analysis. The acid in the samples was neutralized to prevent further hydrolysis. Distilled water was then

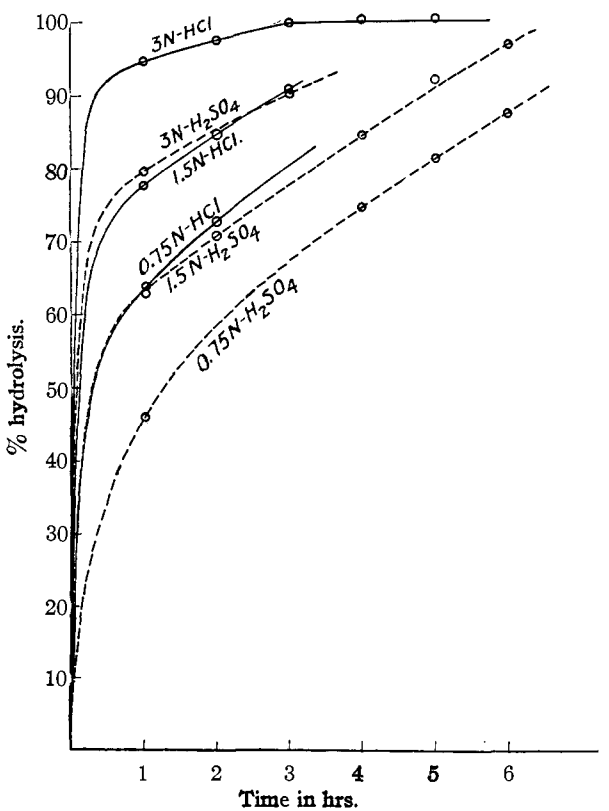

Fig. 2.—Hydrolysis of gelatin at 130°.

TABLE III

HYDROLYSIS OF GELATIN BY HCl AT 130°

Time, hrs.	3.0 *N* HCl: Amino N in 100 cc. of soln., g.	3.0 *N* HCl: Hydrolysis, %	3.0 *N* HCl: *K* Second-order reaction	1.5 *N* HCl: Amino N in 100 cc. of soln., g.	1.5 *N* HCl: Hydrolysis, %	1.5 *N* HCl: *K* Second-order reaction	0.75 *N* HCl: Amino N in 100 cc. of soln., g.	0.75 *N* HCl: Hydrolysis, %	0.75 *N* HCl: *K* Second-order reaction
1	0.1114	94.8	0.182	0.0911	77.6	0.034	0.0727	63.3	0.017
2	.1141	97.3	.183	.0996	84.8	.028	.0843	72.5	.013
3	.1179	100.3	(−)	.1067	90.9	.033			
4	.1187	101.0	(−)					Av.	.015
5	.1178	100.5	(−)		Av.	.032			
		Av.	.183						
	$K/a^{\pm} = 0.045$			$K/a^{\pm} = 0.023$			$K/a^{\pm} = 0.025$		

added to replace the amount withdrawn for samples and the whole procedure repeated. (The dilution of the acid produced by this was not significant, since at the most only 6 cc. was withdrawn from any one flask.)

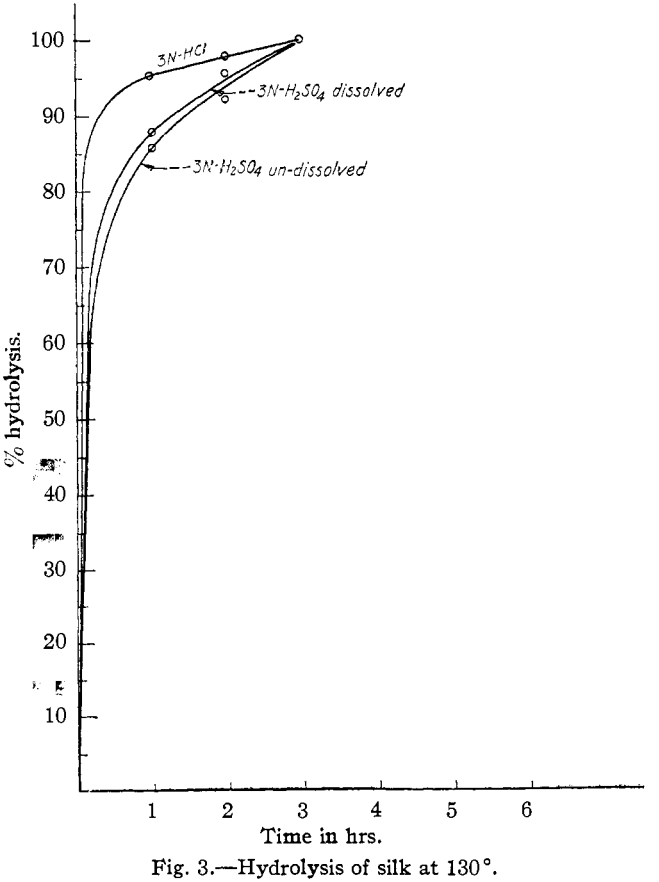

Fig. 3.—Hydrolysis of silk at 130°.

TABLE IV

HYDROLYSIS OF GELATIN BY H_2SO_4 AT 130°

Time, hrs.	3.0 *N* H_2SO_4 Amino N in 100 cc. of soln., g.	Hydrolysis, %	*K* Second-order reaction	1.5 *N* H_2SO_4 Amino N in 100 cc. of soln., g.	Hydrolysis, %	*K* Second-order reaction	0.75 *N* H_2SO_4 Amino N in 100 cc. of soln., g.	Hydrolysis, %	*K* Second-order reaction
1	0.0938	79.9	0.040	0.0744	63.4	0.017	0.0542	46.1	0.0086
2	.1061	90.3	.046	.0833	70.9	.012	...	..	...
4	...	..	..	.0984	84.6	.014	.0878	74.8	.0074
5	...	..	..	...	..	..	.0962	81.9	.0091
6	...	..	..	.1141	97.1	.056[a]	.1032	87.9	.0121
		Av.	.043		Av.	.0144		Av.	.0093
	$K/4a^{\pm} = 0.030$			$K/4a^{\pm} = 0.019$			$K/4a^{\pm} = 0.019$		

[a] Value not used in obtaining average

The dilution of protein due to the replacement of the withdrawn samples with water was taken into account in the calculation of the results. Another source of uncertainty was in the measurement of time, since in the taking of samples the autoclaves had to be cooled and then reheated. As the best thing that could be done under the circumstances, time was

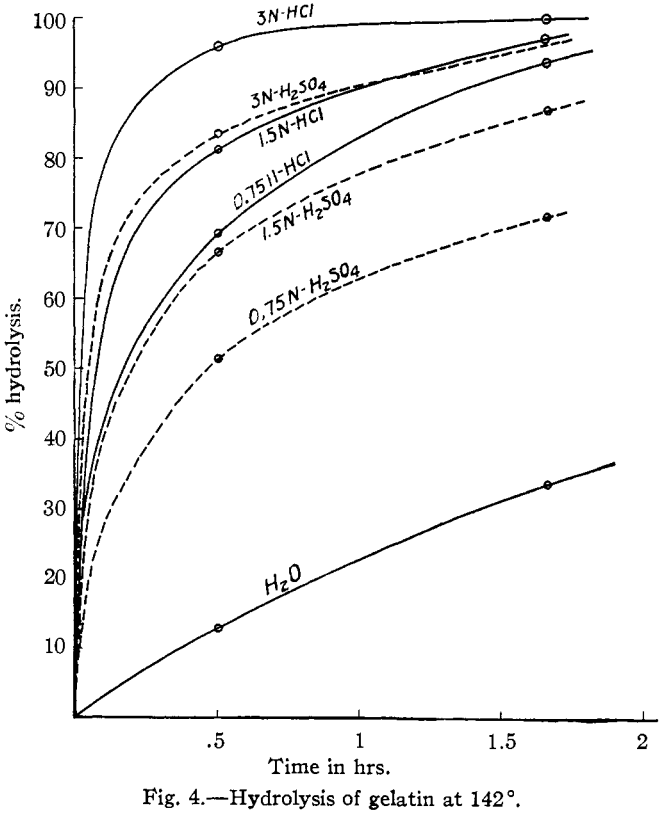

Fig. 4.—Hydrolysis of gelatin at 142°.

measured only when the autoclave was at the desired temperature, the intervals required for heating and cooling being omitted. In the experiments performed at 96–98°, the flasks were kept stoppered, so there was no loss by evaporation and, consequently, the samples for analysis that were withdrawn were not replaced with fresh acid, thus leaving the concentration of protein unchanged through the whole course of the experiment.

TABLE V

HYDROLYSIS OF SILK FIBROIN BY ACIDS AT 130°

	3.0 *N* HCl			3.0 *N* H_2SO_4 undissolved			3.0 *N* H_2SO_4 dissolved		
Time, hrs.	Amino N in 100 cc. of soln., g.	Hydrolysis, %	*K* Second-order reaction	Amino N in 100 cc. of soln., g.	Hydrolysis, %	*K*	Amino N in 100 cc. of soln., g.	Hydrolysis, %	*K* Second-order reaction
1	0.156	95.3	0.20	0.141	86.1	0.062	0.143	87.5	0.070
2	.160	97.5	0.20	.151	92.1	.059	.157	95.6	.109
3	.164	100	..	.163	99.4	..	..	...	..
4	..	...	..	.166	101.0	..	.164	100.0	..
5	..	...	..	.164	100.0	..	.165	100.8	..
6	..	...	..	.166	101.1	..	..	..	..
		Av.	0.20		Av.	.061			
	$K/a^{\pm} = 0.049$			$K/4a^{\pm} = 0.043$					

TABLE VI

HYDROLYSIS OF GELATIN AT 142°

(Time, hrs.)		½			1.66			
Acid, *N*		Amino N in 100 cc. of soln., g.	Hydrolysis, %	*K* Second-order reaction	Amino N in 100 cc. of soln., g.	Hydrolysis, %	*K* Second-order reaction	$K/a^{\pm}$
HCl	3	0.1126	95.8	...	0.1166	99.2	...	...
HCl	1.5	.0940	80.1	0.085	.1136	96.7	0.177[a]	0.061
HCl	0.75	.0816	69.5	.045	.1100	93.6	.088[a]	.076
H_2SO_4	3	.0977	83.1	.098	...	..	...	.068[b]
H_2SO_4	1.5	.0782	66.5	.040	.1020	86.8	.040	.051[b]
H_2SO_4	0.75	.0605	51.5	.021	.0843	71.9	.016	.058[b]
H_2O		.0160	13.6	.0032	.0357	30.4	.0026	

[a] Value not used in obtaining average.

[b] Formula $K/4a^{\pm}$ used for experiments with H_2SO_4.

The increase in the amount of amino nitrogen was taken as a measure of the amount of hydrolysis. The Van Slyke method for amino nitrogen[2] (micro apparatus) was used for the analysis, the reaction vessel being shaken for four minutes. The usual determination and subtraction of the value of the blank were made in each series. A correction was also applied for the lysin nitrogen that is evolved in four minutes, using 6.32%, as the value[3] for the amount of lysin nitrogen present in gelatin and 70% as the fraction of the ε-amino groups[4] that come off on

[2] Van Slyke, *J. Biol. Chem.*, **12**, 275 (1912).

[3] Ref. 1, p. 49.

[4] Dunn and Schmidt, *ibid.*, **53**, 401 (1922).

four minutes' shaking at 23°, the approximate temperature of the room during the course of the analyses. In making the calculations the ash content was also corrected for, so the results are on an ash-free basis.

The silk fibroin used was kindly given to us by Professor C. L. A. Schmidt and had been prepared, free from silk gelatin, by him some years ago. The experimental procedure with the silk fibroin as far as the autoclaving and sampling are concerned is exactly the same as for gelatin at 130°. However, no analysis was made for the ash and also no correction was made for the amount of lysin, the values given for the lysin content of silk fibroin being uncertain. For obtaining the degree of hydrolysis and the calculation of the order of the reaction, the final constant value for the amino nitrogen was taken as the total amino nitrogen content of silk fibroin. The data obtained are presented in tabular form in Tables I to VI and are graphically shown in Figs. 1 to 4.

Discussion of Results

The Order of the Reaction.—Assuming that the increase in amino nitrogen is a measure of protein hydrolysis, calculation of the constants shows that the hydrolyses of both gelatin and silk fibroin are second-order reactions. The values obtained for the constants are given in columns under K in the tables. They are calculated on the basis of per cent. of hydrolysis, the formula used being

$$K = \frac{1}{t} \cdot \frac{x}{(100 - x)} \cdot \frac{1}{100} \qquad (1)$$

where x represents the percentage of protein hydrolyzed in time t. When the hydrolysis is nearly complete the form of the equation is such that any errors in x give disproportionately large fluctuations in the constants. This accounts for some of the fluctuations in the calculated values of K. The tables show that large deviations from the average value of K occur (with a few exceptions) when the hydrolysis is more than 90% complete. Very great accuracy in the experimentally obtained results cannot be expected, first, because of the uncertainty due to the time taken in heating and cooling the samples, and second, because the error of the amino nitrogen method of analysis is not less than 5%. An agreement within 10% in the values of the constant in any one experimental series is the most that can reasonably be expected. In the series at the higher temperatures and concentrations of acid the velocity of reaction was so great that only a very few samples could be obtained at reasonable lengths of time apart, before the hydrolysis was more than 90% complete. On this account the averaged constants in these series are less reliable than in series with more measurements. At this point it is interesting to note that the constants obtained show that silk fibroin, which is one of the most resistant of proteins to solvents, hydrolyzes more rapidly than gelatin, one of the easiest of

proteins to dissolve, or in colloid chemical terminology, disperse. Also, the results show that there is but little difference in the time taken whether undissolved silk fibroin is taken or the silk fibroin is first dissolved in the acid used by boiling under a refluxing condenser until the fibroin is just dissolved. The above also illustrates that aside from the possible theoretical significance of the order of the reaction, the constants obtained are useful in that they are a numerical measure of the relative velocity of hydrolysis.

Besides our own work we made an examination of the data on protein hydrolysis by acids given in the literature to see if they could be subjected to mathematical analysis. Of the results published to date only Carpenter[5] has apparently made any attempt at such a treatment. The most extensive series of measurements of the rate of hydrolysis in the literature

TABLE VII

CALCULATION OF K, VELOCITY CONSTANT FOR A SECOND ORDER-REACTION FROM VICKERY'S DATA ON HYDROLYSIS OF WHEAT GLIADIN AT 96–104°

Time, hrs.	6.02 *N* HCl *K*	Time, hrs.	4 *N* HCl *K*	Time, hrs.	2 *N* HCl *K*	Time, hrs.	1 *N* HCl *K*
1	0.0145	0.5	0.0087	1	0.0028	1	0.00106
2	.0125	1	.0082	3.3	.0019	2	.00065
3	.0134	2.5	.0052	5	.0016	7	.00065
4	.0115	4.5	.0054	7	.0014	12	.00062
5	.0154	5	.0060	9	.0014	18	.00058
7.5	.0149	8	.0063	12	.0014	40	.00056
..	...	13	.0064	17	.0017	69	.00074
..	...	16	.0060	24	.0017	..	....
..	...	24	.0084	45	.0018	..	....
..	...	40	.0078	..	...	..	....
..	...	48	.0055	..	...	..	....
..	...	72	.0091	..	...	..	....
	Av. .0137		Av. .0070		Av. .0015		Av. .00070
$K/a^{\pm}$	= 0.00067	$K/a^{\pm}$	= 0.00095	$K/a^{\pm}$	= 0.00073	$K/a^{\pm}$	= 0.00085

Time, hrs.	0.5 *N* HCl *K*	Time, hrs.	0.2 *N* HCl *K*	Time, hrs.	4 *N* H_2SO_4 *K*	Time, hrs.	0.2 *N* H_2SO_4 *K*
1	0.00045	1	0.00026[a]	1	0.0027	12	0.000043
2	.00035	3.5	.00014	3	.0022	48	.000046
4	.00035	16	.00012	5	.0018	71	.000059
16	.00026	24	.00013	7	.0018	..	.....
22	.00028	40	.00012	11	.0017	..	.....
24	.00028	..	....	17	.0020	..	.....
40	.00033	..	....	24	.0019	..	.....
..	....	..	....	41	.0015	..	.....
..	....	..	....	65	.0019	..	.....
	Av. .00033		Av. .00013		Av. .0019		Av. .000050
$K/a^{\pm}$	= 0.00086	$K/a^{\pm}$	= 0.00081	$K/4a^{\pm}$	= 0.00104	$K/4a^{\pm}$	= 0.00023

[a] Value not used in obtaining average.

[5] Carpenter, *J. Biol. Chem.*, **67**, 647 (1926).

are those of Vickery,[6] who studied the rate of hydrolysis of wheat glindia solutions in various concentrations of hydrochloric and sulfuric acids at temperatures between 93° and 110°. In his report Vickery has made no attempt to calculate the order of the reaction. From the data given we have carried out such calculations and found that the rate of hydrolysis of gliadin in acid solution follows the course of a second-order reaction as do gelatin and silk fibroin—here, too, assuming that the increase in amino nitrogen is a measure of the amount of hydrolysis. The constants calculated from Vickery's data are given in Table VII. It is apparent that the fluctuations in the values of the constants for any series are about the same as for our results. The important thing is that the constants show no particular increasing or decreasing trend, whereas when the formula for some other order is used, for example, unimolecular, the calculated constants show such a trend. Dunn[7] has also measured the rate of hydrolysis of casein at one single acid concentration and temperature. Unlike that of the above-mentioned proteins, the order of the reaction for casein is not bimolecular, but appears to be unimolecular. The constants calculated for a unimolecular reaction from Dunn's data are given in Table VIII. It is interesting to note that Carpenter,[5] working

TABLE VIII

VALUES OF K, VELOCITY CONSTANT FOR A FIRST-ORDER REACTION FROM DUNN'S DATA ON HYDROLYSIS OF CASEIN BY 3.58 N H_2SO_4

Time, hrs.	5	8	10	13	15	16	20
Amino N in % of total minus lysin N	30.5	38.4	49.4	54.3	57.7	62.1	64.3
K, unimol.	0.056	0.052	0.063	0.062	0.066	0.091	..

at temperatures between 5° and 60° and in greatly diluted hydrogen chloride solutions, also found the hydrolysis of casein to follow a unimolecular reaction. The other results on the rate of hydrolysis of proteins to be found in the literature, we were unable to fit to any simple mathematical analysis.

Catalysis of Protein Hydrolysis by Acids.—As is usual in hydrolysis reactions, acids accelerate the rate of hydrolysis. It has been observed that the catalytic effect of the acid was roughly proportional to the concentration and Vickery[6] observed that at the same concentration sulfuric acid is much less effective than hydrochloric. This is also true of our data, sulfuric acid showing only about half the accelerating effect of a similar solution of hydrochloric acid. Quantitatively, in recent years it has been shown that the catalytic effect of acids on the rates of reaction is proportional, not to the concentration of the acid, but instead to the ther-

[6] Vickery, *J. Biol. Chem.*, **53**, 495 (1922).

[7] Dunn, THIS JOURNAL, **47**, 2564 (1925).

modynamic activity of the acid.[8] That this holds true for the influence of acid on the hydrolysis of protein solutions can be shown from Vickery's data and ours. If the velocity is proportional to the activity, then at any one temperature the velocity constant for any acid concentration divided by the activity of the acid should be a constant.

$$K/a^{\pm} = Ka \qquad (2)$$

For hydrochloric acid, using the activity coefficients given by Lewis and Randall[9] for 25° without any corrections for difference in temperature, the constant Ka has been calculated with results as shown at the bottom of each table under the heading $K/a^{\pm}$. Although here again, while the agreement is not as good as would be desirable, it is sufficiently good to indicate the correctness of the proposition. Vickery's extensive series of measurements at different concentrations of hydrochloric acid illustrate this particularly well. Similar calculations were carried out on the results with sulfuric acid. The activity coefficients here, too, were taken from Lewis and Randall[10] and from these values the mean activity of the acid at the desired concentrations was calculated. In some cases interpolated values for the activity coefficients had to be used. The results as for hydrochloric acid are given at the bottom of each table, but under the heading $K/4a^{\pm}$.[11]

In our experiments the values of Ka obtained from the results with sulfuric acid are in substantial agreement with the values obtained with hydrochloric acid. However, the two values calculated from Vickery's results for gliadin are not in as good agreement with the values with hydrochloric acid.

Effect of Temperature.—As part of our plan to be able to predict the course of a protein hydrolysis under the conditions desired, we studied

[8] Harned, THIS JOURNAL, **40**, 1462 (1918). Jones and Lewis, *J. Chem. Soc.*, 117, 1120 (1920).

[9] Lewis and Randall, "Thermodynamics," McGraw-Hill Book Co., New York, 1923, p. 336.

[10] Ref. 9, p. 357.

[11] The necessity for using $K/4a$ follows from the definition of activity by Lewis and Randall [Ref. 9, Chap. XXVI]. This can be illustrated in the following way. In very dilute solutions the activity becomes equal to the molality. Using m to represent molality, then in accordance with the equation of chemical equilibrium, the equation for a binary acid such as hydrochloric is $(m_+m_-)/m = K$. The standard state is then so chosen that the K in the equation becomes unity. The analogous equation for an acid such as sulfuric is $[(2m_+)^2(m_-)]/m = K$, and Lewis and Randall again so choose the standard state as to make K unity. However, to have a comparative standard state for the two types of acid, K instead of being taken as unity for sulfuric acid, should be taken as 4 (where the molality of the acid is used as the unit of concentration). Because of this the mean activity for sulfuric acid calculated from the activity coefficient tables of Lewis and Randall must be multiplied by 4 to give the same value of Ka as does hydrochloric acid.

the effect of temperature on the hydrolysis of gelatin. To determine the mathematical relationship obeyed by the results a graphical procedure was used. As is well known it is the logarithm of the velocity constant, that is, some simple function of the temperature, rather than the velocity constant itself; so by plotting the logarithms of the velocity constants against various functions of the temperature, the particular relation that gives a straight line can be found. Most commonly it is found that plotting log K against $1/T$ gives a straight line, this being the graphical form of the equation proposed by Arrhenius.[12] However, it was found that the points from our results came most closely to falling on a straight line when log K was plotted against T. In plotting, the average of the values of Ka at a given temperature was used. These averages are 0.003 for 97°, 0.027 for 130° and 0.062 for 142°. The line that best fitted these points was drawn and the equation of this line was then determined, being,

$$\log Ka = 0.0287\ T - 5.30 \qquad (3)$$

It is to be remembered that Ka refers to the velocity constant at unit activity and that the temperature, T, is in degrees centigrade. This type of equation for the influence of temperature on reaction velocity is by no means novel, a considerable number of reactions having been found to obey it.[13] We have now all the necessary data for predicting the course of the hydrolysis of gelatin over a wide temperature range and in any acid solution the activity of which is known.[14] From Equation 3 the velocity constant at unit activity at the desired temperature can be determined. This constant Ka multiplied by the activity of the acid to be used, in accordance with Equation 2, gives the velocity constant for that acid concentration, and the velocity constant used with Equation 1 makes it possible to calculate the time that will be required to effect a given degree of hydrolysis.

Summary

1. The hydrolysis of gelatin and silk fibroin by hydrochloric and sulfuric acids at a number of temperatures has been quantitatively studied.

2. The hydrolysis of these proteins as measured by the increase in amino nitrogen is found to conform to the equation for a second-order reaction. From data in the literature it has been shown that this is also true for gliadin but that casein follows a first-order reaction.

3. The catalytic effect of acids on protein hydrolysis has been found to be proportional to the thermodynamic activity of the hydrogen ion.

[12] Arrhenius, *Z. physik. Chem.*, **4**, 226 (1889).

[13] Mellor, "Chemical Statics and Dynamics," Longmans, Green and Co., New York, 1904, p. 392.

[14] Except in quite dilute solutions the activity of the pure acid at that concentration can be used in calculation.

4. A quantitative relationship for the change of reaction velocity with temperature, has been derived from the data for gelatin.

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THE MOLECULAR REARRANGEMENT OF SOME NEW UNSYMMETRICAL HYDRAZINES

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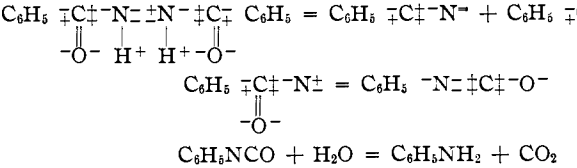
In view of the close similarity between hydroxylamine and hydrazine, particularly in their organic compounds, the probability of rearrangements of hydrazine derivatives analogous to the well-known Lossen rearrangement of hydroxamic acids was predicted by Stieglitz.[1] Subsequent work justified this prediction and several rearrangements of organic hydrazine derivatives have been reported from his Laboratory.[2] It has been shown that the cause of these rearrangements may best be ascribed to the fact that one of the nitrogen atoms of the hydrazine lacks its complete complement of electrons[3] and hence serves as a center of instability. A suitable impulse starts the rearrangement, resulting in this nitrogen atom obtaining its full quota of electrons, probably through the formation of a univalent nitrogen compound in which, as a result of the electron shift, a radical "R" migrates from carbon to the nitrogen atom. Thus, with *sym.*-dibenzoyl hydrazine it[4] was found that the impulse necessary to start the rearrangement was sudden high temperature. When subjected to this treatment the compound was completely decomposed, forming in part very stable five-membered ring compounds and in part undergoing the expected rearrangement. The molecules were here sundered between the nitrogen atoms, one part appearing as the nitrile while the other appeared as *aniline* and *carbon dioxide* (hydrolysis products of phenyl isocyanate), a phenyl group having migrated from carbon to nitrogen. The reaction may be written



$$C_6H_5NCO + H_2O = C_6H_5NH_2 + CO_2$$

or in terms of recent methods of expressing valence,

[1] Stieglitz and Senior, THIS JOURNAL, **38**, 2727 (1916).

[2] Stieglitz and Brown, *ibid.*, **44**, 1270 (1922). J. F. Smith, E. C. Gilbert, E. S. West, etc., Doctor's *Dissertations*, University of Chicago, **1922**, **1922**, **1923**.

[3] Stieglitz, THIS JOURNAL, **44**, 1293 (1922).

[4] Gilbert, Abstract of Theses, *University of Chicago Sci. ser.*, 1, 177–182 (1923).