CLXXI. INVESTIGATIONS ON GELATIN.

PART X. THE EFFECT OF PREVIOUS TREATMENT WITH ACID AND ALKALI ON THE DIAMINO-NITROGEN FRACTION OF THE HYDROLYSIS PRODUCTS OF GELATIN.

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Report to Adhesives Research Committee, Department of
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(Received October 13th, 1927.)

IN these experiments Coignet's Gold Label gelatin purified by electrodialysis was used. Gelatin was allowed to stand in contact with HCl solutions varying in strength from concentrated acid to $N/70$. The most convenient quantity of gelatin for determining the Hausmann numbers by the micro-method [Thimann, 1926] is 0.1 g. of the dry protein, and this amount was taken throughout. After standing for 48 hours at room temperature the solutions were hydrolysed by pouring into boiling acid at such a rate that the boiling was not interrupted. The acid in the flask was of such a strength that the final mixture was approximately 20 %.

A few duplicate experiments were performed allowing a time of 3 days before hydrolysing instead of 48 hours. As the results agreed fairly closely it was assumed that 48 hours was sufficient for equilibrium to be reached.

Table I (see also Fig. 1).

Concentration of HCl solution	Amide-N %	Diamino-N %	Mono-+ non-amino-N %	Total %
0	2.6	17.7	79.6	99.9
0.0143 <i>N</i>	2.4	17.8	79.8	100.0
0.1 <i>N</i>	2.7	18.4	79.1	100.2
0.77 <i>N</i>	2.4	20.6	77.2	100.2
1.4 <i>N</i>	2.3	22.3	75.2	99.8
2 <i>N</i>	2.5	23.0	74.3	99.8
2.3 <i>N</i>	2.4	23.5	73.8	99.7
3.45 <i>N</i>	2.5	22.9	74.6	100.0
5.45 <i>N</i>	2.3	21.3	76.1	99.7
8.8 <i>N</i>	2.2	15.2	82.3	99.7

Effect of NaOH solutions.

A similar series of experiments was carried out with gelatin in the presence of alkali. As the presence of salt influences the diamino-nitrogen precipitated by phosphotungstic acid, control experiments were performed. The hydrolysing acid in these cases contained exactly the same amount of salt as the acid used in the hydrolysis of the protein solutions.

Table II.

Concentration of NaOH	Amide-N %	Diamino-N %	Mono-+ non-amino-N %	Total %
0.1 N	2.2	18.0	79.3	99.5
Control 0.1 N	2.5	18.1	78.8	99.4
N	2.8	21.0	76.7	100.5
Control N	2.6	18.6	78.5	99.7
2 N	2.6	23.6	73.4	99.6
Control 2 N	2.3	19.1	78.9	100.3

Hence the corrected values of diamino-nitrogen due to the influence of NaOH are as follows, the excess over 18 in the controls being subtracted from the experimental numbers:

Normality	0.1 N	N	2 N
Diamino-N	18.0	20.4	22.5

Effect of acid on heated gelatin.

A 10 % gelatin solution was boiled in a steamed-out Jena flask for 2 days; 1 cc. of this solution was allowed to stand with 10 % HCl solution, which is the concentration which gives the greatest rise of diamino-nitrogen with ordinary unheated gelatin.

Gelatin treated with HCl prior to hydrolysis

Fig. 1.

Table III.

	Amide-N %	Diamino-N %	Mono-+ non-amino-N %	Total %
Heated gelatin	2.5	18.5	79.5	100.5
Treated with 10 % HCl	2.6	18.1	79.0	99.7

Effect of pepsin and trypsin.

200 cc. of 4 % gelatin solution in $N/10$ HCl were warmed to 37° and 5 cc. of 1 % pepsin solution added. This mixture was kept at 37° and at intervals 25 cc. were pipetted into a flask, cooled and titrated with $N/10$ NaOH in the presence of formalin. When the formalin titration values became constant a portion of the gelatin solution was hydrolysed and the Hausmann numbers were determined. At the same time a control experiment was set up using the same quantities of reagents but the activity of the pepsin was destroyed by boiling before it was added to the gelatin solution.

A similar experiment was performed with trypsin.

Table IV.

	Amide-N	Diamino-N	Mono-+ non-amino-N	Total
	%	%	%	%
Pepsin	2.9	19.3	77.5	99.7
Control	2.4	19.0	78.2	99.6
Trypsin	2.5	20.0	77.0	99.5
Control	2.6	19.2	78.4	100.2

Hence the corrected values of diamino-N due to enzyme action are as follows:

Pepsin	...	...	18.3
Trypsin	...	...	18.8

The gelatin used had not been freed from the soluble fractions by flocculation, and therefore the numbers in this paper are not absolute; they do, however, indicate the general trend of the action of acids and alkalis on gelatin.

This work was carried out on the suggestion of Prof. S. B. Schryver.

REFERENCE.

Thimann (1926). *Biochem. J.* **20**, 1190.