XIV. THE FORMATION OF A PEPTONE FROM CASEINOGEN BY THE PROLONGED ACTION OF DILUTE HYDROCHLORIC ACID IN THE COLD.

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The partial hydrolysis of proteins by strong acids in the cold was worked out very extensively by Emil Fischer, Abderhalden and their co-workers. As the result of the hydrolysis a number of well defined polypeptides were isolated. Methods were also devised for their separation.

We are not aware however that hydrolysis has been attempted by the prolonged action of weak acids, such for instance as hydrochloric acid in double the concentration in which it occurs in the gastric juice of the dog. The question arose whether hydrolysis took place at all in these conditions and if it did whether the products differed from those obtained by hydrolysis with strong acids.

For the purpose of testing these possibilities commercial caseinogen was left for three months in contact with 1 % HCl-solution. With the aid of the usual methods a product was separated, which, although not quite pure, proved to be a peptone. Although the amount of the substance available for the investigation was small it was possible to determine some of the amino-acids present in the preparation by using the excellent methods brought forward by van Slyke and Folin. As a result of this investigation it was found that the peptone obtained contained only a trace of tyrosine, whereas the peptones separated in this way are usually very rich in this amino-acid. This perhaps suggests that weak acids act on proteins differently from strong acids. A careful control of the commercial caseinogen was made to ascertain whether the product isolated was not present preformed in the commercial preparation, with entirely negative result. This control was performed because it seemed surprising that such weak acids as we had used were able to disintegrate the protein molecule to such an extent.

During this enquiry we had the opportunity to investigate the action of weak acids on the reaction of peptones with triketohydrindene hydrate and we have found that a drop of a decinormal solution of a mineral acid inhibits the reaction completely; the action of organic acids in a ten times higher concentration is the same.

EXPERIMENTAL.

1 kg. of commercial caseinogen was left in contact with 5 litres of a 1% HCl-solution for three months. The suspension of caseinogen which had not been attacked was filtered off. The filtrate which had a deep brownish colour was acid and still contained free hydrochloric acid. The liquid which showed no sign of putrefaction gave a strong biuret reaction, but no Millon reaction. The solution was precipitated in the usual way with a 50% solution of phosphotungstic acid. After 24 hours the precipitate formed was filtered off. The filtrate was still found to give a strong biuret reaction but was not investigated any farther.

The phosphotungstate obtained was decomposed in the usual way with baryta, and the filtrate evaporated *in vacuo* to a syrup which was dissolved in methyl alcohol and filtered into a large volume of absolute alcohol. The product separated was filtered off and washed with alcohol and ether. It was a white powder, fairly soluble in cold water and non-hygroscopic. Yield 14 grm.

The product contained a trace of ash, a trace of sulphur and no phosphorus. It contained 13.41% N by Kjeldahl's method and 2.33% of amino-nitrogen before hydrolysis.

The nature of the product was found to be that of a peptone as shown by the reactions described below:

I.	Millon	...	...	...	Trace.	Traces of tyrosine.
II.	Diazo-reaction	...	...	...	+	
III.	Triketohydrindene hydrate (Ninhydrin)	...	...	...	+	
IV.	Folin's phenol-reagent	...	...	...	Trace.	
V.	Xanthoproteic reaction	...	...	...	+	
VI.	Biuret	...	...	...	Pink-violet.	
VII.	Bromine	...	...	...	-	
VIII.	Glyoxylic acid	...	...	...	+ ¹	
IX.	Diazo-reaction after benzoylation of the product	...	...	...	-.	No histidine.

¹ The glyoxylic acid reagent was prepared following Benedict [1909] by the action of magnesium on oxalic acid.

For the control 100 grm. of commercial caseinogen were extracted for one hour on the shaking machine with HCl of the same concentration as that mentioned above. In contrast to the previous one the suspension

filtered with great difficulty. The filtrate was colourless but slightly opalescent. The solution gave a precipitate with phosphotungstic acid which yielded on decomposition under the same conditions as above 0.4 g. of a white substance which proved to be mostly inorganic; it consisted of ammonia salts chiefly and gave no trace of a biuret reaction. One can conclude therefore that the peptone obtained was formed by the action of the 1% HCl on caseinogen.

With a view to determining some of the amino-acids present in this peptone a complete hydrolysis was performed according to van Slyke's method. An estimation of tyrosine was done separately according to the method of Folin and Denis [1912], but only a trace of this amino-acid was found, due very likely to an impurity. An attempt was made to estimate tryptophane by the recently described colorimetric method of Herzfeld [1913], but the method proved in our case to be unsatisfactory as a decolorisation took place¹.

The result of total hydrolysis of the peptone.

Total nitrogen	13.35 %	Histidine-N.	0
Melanine-N.	0.15 %	Lysine-N.	(?) 5.23 %
Ammoniacal-N.	0.90 %	Cystine-N.	0.30 %
Total amino-N.	5.78 %	Total N. of the phosphotungstic acid	
N. of the phosphotungstic acid ppt.	5.53 %	filtrate	6.16 %
Amino-N. of the phosphotungstic		Amino-N. of the phosphotungstic	
acid ppt.	2.78 %	acid filtrate	1.92 %
Arginine-N.	0	Tyrosine-N.	0.09 %

The results of the hydrolysis seem to suggest that the only hexone base in the product is lysine, the nature of the monoamino-acids being still unknown.

The effect of acids on the reaction of the peptones with ninhydrin.

One drop of a decinormal solution of hydrochloric or sulphuric acid added to 10 cc. of the peptone solution completely inhibits the reaction, both with the peptone described above and also with Witte peptone. The same effect was obtained with one or two drops of a normal solution of acetic or lactic acid.

¹ The same decolorisation took place when we tried to estimate tryptophane in maize by this method.

REFERENCES.

- Benedict (1909), *J. Biol. Chem.* **6**, 51.
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