LXXXIX. THE HYDROLYSIS OF PROTEIN BY A HEAT-STABLE CATALYST PRESENT IN MUSCLE.

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IN a previous publication [Clifford, 1923] a rise of amino-nitrogen was recorded in meat extract and histidine solution, kept constantly at 95–100°, when cod muscle was present, whilst a simultaneous drop in iminazole content occurred. The amino-N rise might have been a simple heat hydrolysis of protein from the added tissue, or like the iminazole fall, the result of a catalytic action. Experiments were therefore carried out with protein solutions kept at 95–100° for various periods of time, and the rise of amino-N estimated by the method of van Slyke. Iminazole estimations [Clifford, 1921, 1] were also performed on the solutions.

Effect of Heat on Gelatin Solution.

A 5 % solution of gelatin was made with distilled water, and 10 cc. of this placed in each of several test tubes. The tubes were then plugged with cotton wool, and left in a constant boiling water-bath for 28–41 days. Water lost by evaporation was replaced each day. When required, a tube was removed, its contents made up to 95 cc. with distilled water, unaltered protein removed by the addition of 5 cc. of 20 % meta-phosphoric acid, and the filtrate used for amino-acid estimations, using van Slyke's micro-apparatus. From the remainder of the filtrate the iminazole content was determined according to the method previously described [Clifford, 1921, 1].

The results are shown in the figure, curves 43 and 54. A slight but definite rise of amino-N occurs, being 27·0 mg. per 100 cc. of gelatin solution in 28 days in Exp. 43 and 30·71 mg. in 41 days in Exp. 54.

The free iminazole content calculated as histidine, in Exp. 54, rose from zero on the first day of the experiment to 4·0 mg. per 100 cc. of solution on the 41st day. This probably was due to histidine liberated during the hydrolysis of the gelatin.

Effect of Heat on Albumin Solution.

A 5 % solution of dried egg albumin in distilled water was treated in a similar manner to that described for gelatin. Again a rise of amino-N took place, though considerably less than with gelatin. The results are shown in curves 47 and 53.

Fig. 1.

From these curves it can be seen that in 47 the rise of amino-N was 7.1 mg. per 100 cc. in 28 days, and in 53 was 13.5 mg. in 41 days.

The iminazole content, calculated as histidine, in 53 rose from zero to 6.0 mg. per 100 cc. in the 41 days. In these experiments the hydrolysis was

less than with gelatin, probably owing to the solubility of the latter, whilst the albumin was a firm solid mass at 95–100°.

Neither of these results is large enough to account for the rise of amino-N in iminazole solutions with muscle described in a previous paper [Clifford, 1923], where 10 cc. of 0.05 % histidine with 5 g. of cod kept in a constant boiling water-bath showed an increase of 43 mg. of nitrogen per 100 cc. in 21 days, and with 5 g. of washed beef showed an increase of 126 mg. in the same time. It seemed from these results that the increase of amino-N must either have been due to the actual structure of the muscle protein which lent itself more readily to hydrolysis than did egg albumin or gelatin, or else due to a catalytic action exercised by some substance in the muscle which accelerated protein hydrolysis at 95–100°.

Further experiments were carried out to determine these points.

Effect of Heat on Cod Muscle in Water.

Because cod muscle contains no extractable iminazole derivatives but at the same time possesses a heat-stable catalyst which destroys iminazole rings and apparently also a catalyst capable of hydrolysing protein, it was chosen as a source of catalyst.

The experiments were carried out as follows:

5 g. of minced cod muscle were placed in each of several test tubes, and about 10 cc. of water were added. The tubes were then plugged with cotton wool and treated exactly as described for gelatin.

Again there was a rise of amino-N, this time considerably greater than for either of the two simple protein solutions. The results are shown in curves 45 and 57.

From these results it appeared that simple hydrolysis could not account for the whole of the nitrogen rise, for since the protein of heated cod muscle more closely resembles coagulated albumin, in its solubility, than a gelatin solution, it would have been expected to show a hydrolysis similar to that of albumin in being less than that for gelatin.

There was a possibility that the extractives from the cod muscle had been split by a catalyst in the muscle as previously shown to be the case with the iminazole ring [Clifford, 1922], and the extra nitrogen derived in this way, or there might have been an acceleration of protein hydrolysis. It was also possible that both of these actions took place simultaneously. No diazotisable iminazole was found in the protein-free filtrate during the experiment, but a yellow colour was obtained similar to that previously found with cod muscle extracts [Clifford, 1921, 2].

Experiments were carried out to determine the effect of cod muscle on the hydrolysis of added protein. For this purpose gelatin and egg albumin were used.

Effect of Cod Muscle on Heated Gelatin Solution.

5 g. of minced cod muscle were placed in 10 cc. of 5 % gelatin solution in each of several test tubes, and these were treated as described for the former experiments. The rise of amino-N which occurred was found to be greater than the sum of the results obtained for gelatin alone and for cod and water alone. The results are given in curves 44 and 55.

Experiment 44 showed a rise of 197.3 mg. of amino-N per 100 cc. of solution in 28 days; and Exp. 55 a rise of 255.4 mg. in 41 days.

Thus there was an excess of 54.15 mg. in one case, and of 152.19 mg. in the other, over the nitrogen liberated from gelatin and from cod separately in a similar period of time. Apparently there is a heat-stable catalyst in cod muscle which can hydrolyse gelatin.

There was an absence of free iminazole in the protein-free filtrate all through the experiment as opposed to the slight rise in the case of gelatin alone. This was expected from earlier observations on the destruction of carnosine and histidine by cod muscle at 100° [Clifford, 1922, 1923]. The observations were then extended to egg albumin.

Effect of Cod Muscle on Heated Egg Albumin.

Experiments were carried out substituting 5 % egg albumin for the 5 % gelatin, and again adding 5 g. of minced cod muscle to every 10 cc. of protein solution.

A rise of amino-N greater than those from the two substances kept separately again occurred, but it was not so marked as with gelatin. The results are shown in curves 46 and 56.

In 46 there was a rise of 107.7 mg. per 100 cc. of solution in 28 days, and in 56 a rise of 134.2 mg. in 41 days. The excess of amino-N over the sum of the results from albumin alone and cod alone in these two experiments was 44.45 mg. and 48.2 mg. per 100 cc. solution respectively. The cod muscle catalyst is therefore capable of hydrolysing egg albumin.

Again there was no diazotisable iminazole all through the experiment (56), any liberated histidine being destroyed by the catalytic action of the cod muscle.

DISCUSSION OF RESULTS.

There is a small but definite hydrolysis of protein, when a simple watery solution of gelatin or egg albumin is kept at 95–100° for 28–41 days.

This rise of amino-N is greater, when, in place of gelatin or egg albumin, cod muscle is substituted. In order to account for the increase, the muscle must be supposed to contain a substance capable of splitting protein or other nitrogenous matter present in muscle, with the liberation of free amino-groups.

When cod muscle is added to gelatin or to egg albumin solution, the rise of amino-N is considerably greater than that obtained by adding the results

of protein solution alone, to that of cod and water alone. Since in the cod and water, and in the cod and protein experiments there was an equal amount of cod muscle and therefore of extractives, it is reasonable to suppose that the increase of amino-N must have come from the hydrolysis of the extra protein in the gelatin and albumin tubes.

The fact that with albumin the rise of nitrogen is less than with gelatin can probably be accounted for by the insolubility of egg albumin at 100° while at that temperature gelatin is soluble, and more easily attacked by catalysts.

Free histidine naturally appears in the protein-free filtrate after the simple heat hydrolysis of protein. If cod be present, this iminazole is destroyed by the catalytic action of the muscle. The yellow colour obtained in the place of the red of diazotised histidine deepens throughout the experiment.

From these results it seems that there is a heat-stable catalyst in cod muscle which can hydrolyse protein, and gives a curve of the same type as that obtained for the destruction of iminazole if inverted [Clifford, 1922, 1923].

Experiments are in progress with the object of isolating this catalyst and freeing it from muscle proteins when it should be possible to decide whether the catalyst which hydrolyses proteins is identical with that which destroys the iminazole ring.

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