LXIX. A HEAT-STABLE CATALYST IN ANIMAL TISSUES WHICH DESTROYS THE IMINAZOLE RING AND UNMASKS AMINO GROUPS.

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(Received June 21st, 1923.)

AN account of the catalytic destruction of carnosine *in vitro* at 100° by a substance present in skeletal muscle and liver has been previously published [Clifford, 1922]. These experiments showed a progressive loss of the iminazole ring when a solution containing carnosine was kept with skeletal muscle or liver over a period of 3–4 weeks. The amount of iminazole was estimated by a colorimetric method described previously [Clifford, 1921, 1], depending on the coupling of the histidine molecule of carnosine with diazo-reagent. The loss of red colour could be accounted for in two ways. The ring substance might have been joined to others in a more complicated manner, similar to that of the histidine in proteins, and thus be unable to couple with the reagent. Another and more probable solution was that the catalyst actually broke up the ring into some simpler substance. In the latter case it was possible that the nitrogen of the ring would be converted to amino nitrogen and then the increase of amino nitrogen could be estimated by van Slyke's method. Accordingly experiments were carried out in which parallel estimations of diazotisable iminazole and amino nitrogen were made.

Iminazole decrease and Amino N increase in Beef and Water at 100°.

3–5 g. of finely minced beef were placed in test-tubes with about 15 cc. water and the tubes were plugged with cotton-wool and left in a constantly boiling water-bath for 17–24 days. Water was added to the test-tubes when necessary to make up for that lost by evaporation.

At intervals a tube was removed, about 30 cc. water and 5 cc. of 20 % metaphosphoric acid added and the whole made up to 50 cc. and left to stand 12–24 hours. After filtration part of the protein-free solution was tested colorimetrically for carnosine [Clifford, 1921, 1] and van Slyke estimations for amino acids were performed on the other portion, the micro-form of the apparatus being used.

The results are shown in Fig. 1, Curves 32 and 41.

From these results it can be seen that in both cases a three-stepped curve was obtained but in opposite directions, *i.e.* a fall of iminazole was accompanied by a rise of amino acid in the protein-free extract. The rise of amino N is, however, above that which could come from carnosine in the solution and

Fig. 1.

thus must come from a hydrolysis of the beef protein itself, or from some nitrogen-containing extractives. These substances are affected in a similar way to the iminazole ring of carnosine and also give a rise of amino N in the same three-stepped manner.

That there are considerable amounts of other soluble extractives giving amino N can be seen from the first figures of Expt. 32 where with meat extract containing carnosine to the extent of 910 mg. per 100 g. meat, 119 mg. amino N per 100 g. were present in place of 52 mg. if the whole came from carnosine. In the case of Expt. 41 again there are 187 mg. per 100 g. instead of 48 mg. if due entirely to carnosine.

The fall of carnosine and rise of amino N is against the findings of Baumann and Ingvaldsen [1918] who found muscle and liver extract to be without any hydrolytic action on carnosine. Their experiments, however, assuming enzyme action, were carried out at 40° for a period of 24 hours only.

In one of the author's experiments in which meat and water were sterilised by boiling for 30 minutes and then left at 37° no change in carnosine content was observed in 13 days, so it is unlikely that at 40° any change should take place in 24 hours.

No rise in amino N with Beef Extract.

It was thought possible from the above experiment that the rise in amino N might be due to hydrolysis of soluble protein in the extract, though seven previous experiments [Clifford, 1922] showed that in an extract kept for 13–16 days no change in carnosine content occurs.

Therefore 10 cc. of an extract made by boiling 60 g. of beef in two separate portions of 150 cc. water were placed in each of a series of test-tubes, plugged and left at 100°.

Estimations of carnosine and amino N were carried out on these at intervals. The carnosine remained constant, and the amino N showed an insignificant rise of 4 mg. per 100 g. meat extracted, whilst in the previous experiment the rise was 158 mg. per 100 g. (Fig. 1, 38.)

It is possible that the very small protein content of these tubes compared with that of those in which 5 g. of meat including its fibrin remained in a small bulk of water could account for the difference. This is improbable but experiments with strong protein solution in water at 100° are in progress to ascertain if a large rise of amino N in a three-stepped curve will result.

The heat-stable catalyst responsible for the results obtained above is not confined to muscle tissue as the following experiments will show.

Rise of amino N and Decrease of Iminazole with Liver in Meat Extract.

To separate test-tubes, each containing 10 cc. of beef extract made by boiling minced beef with water and filtering, 5 g. of calf liver were added. These tubes were plugged and placed in the water-bath and treated in the same way as the beef and water tubes. Again there was a rise in amino N and a fall in carnosine content, both with the usual curve. The rise of amino N once more was greater than could be accounted for by the conversion of the diazotisable N to amino N (Fig. 1, 40), indicating the presence of other substances than carnosine, which are split by the same agent in a similar way to carnosine.

The question then arose as to whether the catalyst would destroy iminazole compounds other than carnosine. Histidine was therefore used in place of muscle extract and the following experiments were carried out.

Effect of Cod Muscle on Histidine Solution.

Cod muscle has previously been shown to be free from any iminazole substance capable of coupling with diazo-reagent [Clifford, 1921, 2]. It has also been shown to contain a catalyst which destroys carnosine [Clifford, 1922], and was therefore used to determine if the catalyst could act on a simpler iminazole compound.

Separate tubes, containing 10 cc. of 0.05 % histidine monohydrochloride solution, were taken and 5 g. of minced cod muscle were added. These tubes were treated in the same way as those with beef and water. Again the iminazole which could be diazotised was destroyed, and there was a rise of amino N. This was greater than could have come from the histidine and therefore must have come from the cod. At the end of 21 days there was a loss of histidine which could account for a rise of 9.9 mg. of nitrogen if all the ring N were converted to NH_2 , whilst the actual rise was 43 mg.

However, the shape of the curves was the same as with carnosine in meat extract, both with regard to iminazole fall and to amino N rise (Fig. 2, 35). From this it would appear that the catalyst will attack histidine in the same way that it will attack β -alanyl-histidine and may be capable of splitting any iminazole ring.

Effect of Liver on Histidine Solution.

The experiment was carried out as for cod, except that 5 g. of minced calf's liver was used in place of cod muscle, again with the idea of introducing a tissue containing the catalyst which at the same time was devoid of free iminazole. The result was similar to that obtained with cod, namely, a fall of iminazole giving a red colour when diazotised, and a large rise of amino N (Fig. 2, 36).

The rise of amino N here is 42 mg. whilst the most which could have come from the destroyed histidine is 8 mg.

Effect of Washed Meat on Histidine Solution.

It has previously been shown [Clifford, 1922] that meat which has been minced and washed in running water for 24 hours still contains the catalyst, though obviously devoid of all water-soluble substances. Accordingly 5 g. of minced beef washed for 24 hours were added to another series of tubes containing 10 cc. of 0.05 % histidine monohydrochloride solution. On repeating estimations as for the cod muscle and calf liver series similar results were obtained. There was a still greater rise of amino N, it being 126 mg. at the end of 21 days against a possible 9.2 mg. from histidine nitrogen (Fig. 2, 37).

That the destruction of the histidine ring is not mere hydrolysis at 100° is shown by the following experiment.

Effect of Temperature on Histidine Solution.

Tubes containing 10 cc. of 0.05% histidine monohydrochloride solution were placed in the bath at 100° and used as controls. In 21 days there was no alteration either in iminazole content as estimated colorimetrically or in amino N as estimated by the method of van Slyke.

0-3-5-9-17-21 days; histidine 50 mg.; amino N 3.7 mg. (Fig. 2, 34).

From this experiment it appears that there is no spontaneous splitting of the histidine ring when a weak solution is kept at 100° constantly for 3 weeks.

Fig. 2.

DISCUSSION OF RESULTS.

The catalytic agent present in beef and cod muscle and in liver is capable of destroying carnosine in a beef extract, and also of destroying histidine in watery solution. This is shown by a decrease of colour on diazotising histidine solution or muscle extract at intervals after the addition of cod, washed beef or liver, the tubes being kept at 100°. This may indicate the existence of a catalyst capable of splitting any iminazole ring.

The peculiar shape of the curve previously observed in the disappearance of carnosine is maintained with histidine solution. At the same time that there is a fall in iminazole there is a rise in amino N which gives a curve in the opposite direction to the iminazole fall. This suggests an opening of the iminazole ring with the changing of its ring nitrogen to amino N.

The rise of amino N is greater than can be accounted for by the opening of the diazotisable iminazole ring and therefore it must in part come from some other source. The protein of the added substance (muscle or liver) may be hydrolysed with the consequent freeing of amino groups. This is improbable as a watery meat extract which necessarily contains some protein can be kept 17 days with a rise of only 4 mg. per 100 g. extracted meat and the iminazole content remains unchanged. Another objection to the simple hydrolysis of protein as a cause of the rise of amino N is the peculiar shape of the curve obtained. This is the converse of that of loss of diazotisable iminazole and suggests a connection between the two changes. This is shown well in Fig. 3, Expt. 35, where the scale has been enlarged.

Fig. 3.

The catalyst may be able to attack other iminazole compounds, so that other extractives besides carnosine may give rise to the extra amino N. There is a possibility that purine bodies such as xanthine and hypoxanthine may have their iminazole group split off as urea, and this could account for discrepancies between ingested purine and excreted uric acid in the body. Creatine is, through guanidine, allied to arginine, and also is known to give rise to creatinine spontaneously when boiled. Creatinine shows the iminazole ring formation and therefore is allied to histidine and the purines. The breaking

of the creatinine ring may be possible by the action of the catalyst destroying histidine, when creatine might result. This would give possibilities of a link between arginine and histidine and their interchangeability in the body.

Experiments are in progress in order to enquire into these various possibilities.

Thanks are due to Professor V. H. Mottram for his advice and interest in the work.

The expenses of the research were defrayed by a grant from the Medical Research Council.

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