XCVIII. THE HEAT COAGULATION OF EGG-ALBUMIN.

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It has recently been suggested by Wu and Wu [1925] that tyrosine is split off when egg-albumin is coagulated by heating. This observation seemed quite probable since it is known that tyrosine is very readily eliminated from proteins. For instance, Fürth and Fischer [1924] found that tyrosine was among the first products of the tryptic digestion of caseinogen and fibrin. As the experiments of Wu and Wu were not decisive it was resolved to repeat and amplify them.

The Weiss [1919] modification of the Millon reaction seemed to offer the most specific and delicate independent test for tyrosine and could be used to detect 1 part of tyrosine in 500,000 parts of solution. It was also decided to coagulate the albumin solutions only partially in order to avoid, as far as possible, the danger of secondary hydrolysis. The total nitrogen determinations were carried out by Pregl's [1924] modification of Parnas and Wagner's micro-Kjeldahl method which will measure 0.02 mg. of nitrogen with certainty.

The crystalline egg-albumin was prepared from fresh hen's eggs in the usual way and recrystallised twice: it was then dialysed in presence of a little thymol until free from ammonium sulphate. A minimal amount of acetic acid was added near the end to aid the removal of ammonia.

2 cc. of albumin solution containing 4.9 mg. of nitrogen per cc. were diluted with 10 cc. of water and heated for 15 to 30 minutes at 60° or 70° and the protein matter precipitated by 15 cc. of diluted "dialysed iron" solution and a trace of potassium sulphate. 5 cc. of the filtrate were used for the nitrogen determination and 3 cc. for the tyrosine test.

Although it should have been possible to detect, by the Weiss reagent, one-tenth of the tyrosine present in the albumin if it had been split off on coagulation, no trace of it could be found. Further, the amounts of total nitrogen found in the filtrate were very small and constant within the limits of experimental error. They were of the order of 0.5 % of the nitrogen used and were almost certainly due to traces of ammonia from the ammonium sulphate used in the preparation of the albumin. They tended to decrease with the longer times of heating as if the substance were adsorbed on the

coagulum. The total nitrogen determinations are not sufficiently delicate to prove the absence of tyrosine but they do show that there is no general breakdown of the molecule under the conditions of the experiments. Exactly similar results were obtained when gradually increasing amounts of (1) acetic acid, (2) caustic soda, were added until there was no coagulum on heating.

As these results were directly opposed to those of Wu and Wu, their experiments were repeated using a thymol-free solution, since the Folin reagent gives a strongly positive reaction with thymol. 20 cc. of 1 % albumin solution and 25 cc. of water were heated in boiling water for 15 minutes, cooled and precipitated with 3 cc. of 2N/3 sulphuric acid and 3 cc. of 10 % sodium tungstate solution. 0.25 cc. of the Folin and Denis [1912] phenol reagent and 3 cc. of 20 % sodium carbonate solution were added to 35 cc. of the filtrate and colour comparisons made with a 1/20,000 tyrosine solution. The experimental values are expressed as mg. of apparent tyrosine per g. of albumin.

	Nitrogen % total used	"Tyrosine" F and D	Increase "Tyrosine"	Tyrosine Weiss
Unheated	0.96	6.1	—	—
15 mins. 100°	0.71	5.6	-0.5	-ve
Unheated	—	8.6	—	—
15 mins. 100°	—	6.8	-1.8	-ve
15 mins. 70°	—	7.7	-0.9	—
15 mins. 60°	—	7.0	-1.6	—
Unheated	—	8.2	—	—
15 mins. 100°	—	7.2	-1.0	-ve
15 mins. 100°	—	8.2	0	-ve

The Molisch carbohydrate test was also negative.

These results are entirely contrary to those of Wu and Wu, in that a decrease in the colour of the phenol reagent is observed in all cases. This decrease is probably due to adsorption of the colour-producing substance on the coagulum.

If the colour was due to tyrosine, the Weiss-Millon reagent should have given a strong positive result. The absence of any colour with this reagent proves that tyrosine is not liberated from albumin on coagulation. Further, the nitrogen determinations show that no measurable amount of nitrogenous substance is liberated at all. If half a per cent. of the total nitrogen in the albumin had been split off it would be detectable by the micro-Kjeldahl method used.

It was possible that any tyrosine liberated would be adsorbed on the coagulum or protein precipitate, so 5 cc. of 1/10,000 standard tyrosine solution were added to 10 cc. of 1 % albumin and 10 cc. of water and the determinations repeated.

	"Tyrosine" F and D	Increase "Tyrosine"
Tyrosine added	5.0	—
Unheated	6.25	—
15 mins. 70°	6.95	+0.70
15 mins. 100°	7.05	+0.80

This clearly indicates that tyrosine is not adsorbed on the coagulum since with increasing time of heating and corresponding increase in the amount of coagulum the tyrosine found does not decrease. The figures obtained are practically identical with those without any addition of tyrosine so that the effect of further additions was examined, the solutions being heated in boiling water for 15 minutes.

5 mg. of tyrosine added	7.25 mg. "tyrosine" found
10 " "	15.0 " "
15 " "	21.5 " "

In all these cases tyrosine can easily be detected by the Weiss test. The first 5 mg. or so of tyrosine added have some action on the chromogenic substance already present but the nature of this action has not been determined.

The nitroprusside tests, as modified by Walker [1925], gave an immediate coloration with any of the filtrates and it was shown that, while cystine gave no colour with the Folin and Denis reagent, an intense blue colour was given by cysteine. It is accordingly suggested that some substance containing a thiol group is liberated when albumin is precipitated by means of sodium tungstate or ferric hydroxide and that this sulphur-containing substance reduces the phenol reagent. It was shown that potassium permanganate was decolorised by the filtrate.

It cannot be too strongly emphasised that the delicate phenol reagent of Folin and Denis may only be used to estimate tyrosine in the proved absence of all readily oxidisable substances, as any such substance will produce the blue colour [cf. Gortner and Holm, 1920].

It does not necessarily follow that the production of this reducing substance involves any chemical decomposition in the present case as it may well be due to desorption accompanying precipitation.

It may be noted that fresh egg-white does contain free tyrosine which collects at the anode in large quantities when an egg-white solution is electrolysed. It may also be detected in the mother liquors after precipitating the crystalline egg-albumin. Tyrosine may thus be found in a badly prepared specimen of albumin. A normal albumin solution which has just commenced to become foul will also contain free tyrosine and the amount of this free tyrosine undoubtedly does increase when the solution is heated.

Sörensen and Sörensen [1925] have also come to the conclusion that heat coagulation is unaccompanied by decomposition but it has not been possible to consult the original paper.

SUMMARY.

1. No evidence can be obtained that tyrosine is split off when albumin is coagulated by heating.

2. A reducing substance is present in the filtrate when an albumin solution is precipitated by sodium tungstate or colloidal ferric hydroxide. This substance probably contains a free —SH group.

3. The Folin and Denis phenol reagent cannot be used to estimate tyrosine in the presence of easily oxidisable substances.

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