THE ENZYMATIC HYDROLYSIS OF RAW AND
HEAT-TREATED EGG WHITE

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The effect of a preliminary heat treatment of proteins upon their digestion in vitro appears to be dependent to some degree on the nature of the protein. The studies of Waterman and Johns (1) and of Jones and Waterman (2) have demonstrated that a partial cooking of phaseolin, casein, and cottonseed globulin increases the extent of hydrolysis of these proteins by the successive action of pepsin and trypsin. The digestion of arachin, however, was uninfluenced by heat treatment prior to enzymatic action.

The question of the relative physiological value of raw and of heat-treated egg white has served to emphasize again the importance of heat as a factor in determining the extent of utilization of proteins. Investigations dealing with this subject have in the majority of instances involved growth or nitrogen balance studies; conflicting results have been reported (3–7). Of particular interest are the recent studies of Parsons and coworkers (8–10) and of Salmon and Goodman (11) on the nutritional disorder produced by feeding large quantities of raw egg white to rats.

In view of the conflicting results and diverse explanations concerning egg white in nutrition, an investigation of the in vitro hydrolysis of raw and of heat-treated egg white has been conducted. Data have been obtained concerning: (1) the comparative rates of digestion of raw and of heat-treated egg white in vitro, and (2) the extent of hydrolysis of both types of substrate by pepsin and trypsin acting both individually and successively.

EXPERIMENTAL

A stock egg white powder was prepared by drying the whites of fresh eggs in vacuo over sulfuric acid at 25°; the dried product
was ground to a fine powder and thoroughly mixed. Standard egg white solutions were prepared by suspending 5 gm. of the powder in 500 cc. of water, shaking until a uniform suspension was obtained, and then slowly adding with shaking 450 cc. of boiling distilled water. The mixture was thoroughly agitated until solution was practically complete, cooled to room temperature, and diluted to 1000 cc. with distilled water. 100 cc. aliquots were withdrawn; for purposes of reference, these will be called Samples A, B, C, D, etc.

Aliquot Sample A was adjusted to pH 8.0 with 1 per cent sodium hydroxide, warmed on a water bath to 40°, and treated with 10 cc. of a 1 per cent trypsin2 solution. The flask was shaken thoroughly and a 10 cc. portion withdrawn for analysis. To the remainder of the solution were added 5 cc. of a 0.4 per cent thymol solution in ethyl alcohol and the flask was stoppered and placed in an incubator maintained at 38°. Samples were withdrawn at varying intervals of time for analysis. Enzymatic action in each withdrawn aliquot was terminated by heating the solution for 5 minutes at 80°. The sample was then allowed to cool to room temperature and titrated by means of the Sörensen formol titration (12).3

A second 100 cc. aliquot (Sample B) of the egg white solution was immersed in a boiling water bath for exactly 1½ minutes.4 In a similar manner, subsequent aliquots (Samples C, D, E, etc.) were heated for 5, 10, 30, 45, 60, and 120 minutes respectively. Each of these solutions was then made up to the original 100 cc.,

1 A small, barely perceptible amount of material failed to dissolve.
2 1:110 trypsin, Difco Laboratories.
3 A series of check determinations has been conducted in which the increase in carboxyl groups was determined by titration in 90 per cent alcohol according to the method of Willstätter and Waldschmidt-Leitz (13), with thymolphthalein as the indicator. The trend of the values given by the latter procedure was similar to that yielded by the Sörensen method. Since the results in these experiments are of interest because of their relative, rather than their absolute magnitude, we are reporting the titration values obtained with the Sörensen technique. The significance of the measurements by each of these two volumetric methods, and the adequate accuracy of the Sörensen procedure for the present type of investigation, have recently received excellent discussion by Richardson (14).
4 The egg white solution became slightly opalescent when subjected to the heat treatment, but there was no flocculation of protein.
replacing the water lost by evaporation during the boiling process. These aliquots were then treated with trypsin, incubated, and analyzed at intervals, as described above. For each 100 cc. aliquot, simultaneous control experiments were performed by incubation of (1) 0.1 gm. of trypsin dissolved in 100 cc. of water and (2) 100 cc. of stock egg white solution; both controls were adjusted to pH 8.0, and analyzed at the same intervals as were the withdrawn samples of the experimental digest. The sum of the two titration values from these control solutions was considered as a blank and subtracted from the figure obtained by analysis of the digest aliquot. Table I presents the experimental values of these trypsin studies.

**Table I**

*Hydrolysis of Egg White by Trypsin*

Each value represents the corrected cc. of 0.05 N sodium hydroxide required to titrate a 10 cc. portion of the digest.

<table>
<thead>
<tr>
<th>Preliminary heating period</th>
<th>Incubation time, in min.</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<tbody>
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<td></td>
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<td>40</td>
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<td>90</td>
<td>120</td>
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<td>300</td>
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<td>min.</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.09</td>
<td>0.11</td>
<td>0.11</td>
<td>0.15</td>
<td>0.17</td>
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<tr>
<td>1.5</td>
<td>0.14</td>
<td>0.26</td>
<td>0.28</td>
<td>0.36</td>
<td>0.44</td>
<td>0.52</td>
<td>0.58</td>
<td>0.68</td>
</tr>
<tr>
<td>5</td>
<td>0.28</td>
<td>0.50</td>
<td>0.56</td>
<td>0.72</td>
<td>0.82</td>
<td>0.95</td>
<td>1.10</td>
<td>1.15</td>
</tr>
<tr>
<td>30</td>
<td>0.48</td>
<td>0.62</td>
<td>0.80</td>
<td>0.82</td>
<td>0.95</td>
<td>1.06</td>
<td>1.12</td>
<td>1.22</td>
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</table>

Experiments were also conducted in which the action of trypsin was preceded by an incubation period with pepsin. In these experiments, 100 cc. aliquots (Samples A, B, C, etc.) of a stock solution of 0.5 per cent egg white powder in 0.3 per cent hydrochloric acid were used. Sample A was treated with 10 cc. of a 1 per cent solution of pepsin,\(^5\) preserved with thymol, and incubated at 38° for 90 minutes. At the end of this period, a 10 cc. aliquot was withdrawn for analysis; the remaining contents of the flask were adjusted to pH 8.0 with \(N\) sodium hydroxide and treated with trypsin, incubated, and analyzed at intervals as already described. Simultaneous blank determinations were conducted on egg white in 0.3 per cent hydrochloric acid and on the enzyme solutions.

\(^5\) 1:3000 pepsin, Parke, Davis and Company.
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The experimental values obtained were corrected by these blank titration values.

Aliquot Samples B, C, D, etc., were subjected to preliminary heating periods of varying length, before being treated with pepsin. The subsequent procedure was identical with that outlined in the preceding paragraph. The results of the studies in which trypsin was preceded by pepsin are presented in Table II.

Under the experimental conditions employed, the cleavage of raw egg white by pepsin is negligible, while trypsin exhibits a limited ability to hydrolyze this substrate. However, despite the apparent inactivity of pepsin with the raw egg white, it appears that this enzyme did cause some change in this material, since trypsinic action was much more extensive when preceded by pepsin than when trypsin alone was used (compare Table I and Table II, raw egg white).

A preliminary heating of the egg white solution prior to incubation was found to be of value in augmenting the activity of trypsin. Table II presents the results of a study in which trypsin was preceded by a 90 minute incubation period with pepsin.

**Table II**

Hydrolysis of Egg White by Trypsin Following a 90 Minute Incubation Period with Pepsin

Each value represents the corrected cc. of 0.05 N sodium hydroxide required to titrate a 10 cc. portion of the digest.*

<table>
<thead>
<tr>
<th>Preliminary heating period min.</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>180</th>
<th>240</th>
<th>300</th>
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<tr>
<td>0</td>
<td>0.20</td>
<td>0.43</td>
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<td>1.06</td>
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<td>1.5</td>
<td>0.62</td>
<td>0.81</td>
<td>0.85</td>
<td>0.92</td>
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<td>1.32</td>
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</tr>
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<td>5.0</td>
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<td>1.27</td>
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<td>1.42</td>
<td>1.54</td>
<td>1.58</td>
</tr>
<tr>
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<td>1.19</td>
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<tr>
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<td>1.54</td>
<td>1.68</td>
<td>1.80</td>
<td>1.80</td>
<td>2.02</td>
<td>2.08</td>
<td>2.12</td>
</tr>
</tbody>
</table>

* The values have been obtained by subtraction of the titration figure found at the end of the 90 minute incubation period for pepsin from the titration value resulting after trypsinic incubation. These pepsin values, expressed as cc. of 0.05 N sodium hydroxide required to titrate 10 cc. of the digest, were 0.0 cc. for raw egg white, and 0.28, 0.42, 0.70, and 1.50 cc. for egg white subjected to preliminary heating periods of 1.5, 5, 30, and 45 minutes respectively.
tion with the enzymes resulted in a marked increase in the extent of hydrolysis of the substrate. When trypsin alone was used, the degree of hydrolysis of egg white increased as the preliminary heating period was extended to 30 minutes. Longer heating periods did not further the hydrolytic process; the titration data for egg white solutions heated for 30, 45, 60, or 120 minutes previous to incubation with trypsin are identical within the limits of experimental error. When the action of trypsin was preceded by pepsin, there was a continued increase in the magnitude of hydrolysis of egg white as the preliminary cooking period was lengthened to 45 minutes. Egg white solutions heated for 45, 60, or 120 minutes previous to peptic plus tryptic action yield similar data.

DISCUSSION

The results which have been obtained are in harmony with those of in vivo experiments which have indicated a better utilization of heat treated than of raw egg white. Of the hypotheses which have been offered to explain this difference in physiological availability, the suggestion that the indigestibility of raw egg white is due to a heat-labile, antitryptic factor appears to be in concordance with the present findings. The recent work of Balls and Swenson (16) on the antitrypsin of egg white is of considerable interest in this connection. These investigators reported the preparation from egg white of a concentrate which inhibits the proteolytic activity of trypsin; the product was described as moderately heat-stable and destroyed by continued boiling. It appears quite possible that under the experimental conditions herein employed the shorter boiling intervals brought about a partial inhibition of the antitrypsin of egg white, while the longer periods of cooking effected a complete destruction of this antienzyme. It is likely, however, that the reported poor utilization and evident toxicity of raw egg white is not due solely to an antitrypsin. The physical characteristics of the material per se may be a contributing factor. It seems significant that the lack of nutritional value of the uncooked material may be related to the limited time which it remains in the stomach (17–19). This shorter period would tend to restrict the action of pepsin; the important rôle of this gastric

7 For an excellent discussion of possible factors involved in the utilization of raw egg white, see reference (3).
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enzyme in the proteolysis of egg white is indicated by the results reported in this communication. In fact, a preliminary treatment with pepsin compares favorably with a 30 minute period of cooking of the egg white in its ability to augment the action of trypsin (compare Table I, 30 minute preliminary heating period, with Table II, no preliminary heating period).

The recent report by Calvery (20) of the extensive action of pepsin on crystalline egg albumin is of interest here in view of the conclusion by Bateman (3) that of the individual proteins constituting egg white, the albumin fraction appears to be the indigestible component. These results differ from those described in this communication. However, the substrate utilized by Calvery was crystalline egg albumin; the preparation of this material in a highly purified state might readily eliminate any enzyme-inhibiting factor present in the native egg white. Furthermore, in the experiments of Calvery 2.5 gm. of enzyme were employed for 1 gm. of egg albumin, whereas in the present investigation only 0.2 gm. of the proteolytic agent was used for each gm. of substrate.

SUMMARY

1. Pepsin produced no significant splitting of raw egg white under the experimental conditions employed. The hydrolysis effected by trypsin was slight; a preliminary incubation of the raw egg white with pepsin greatly facilitated the attack of the material by trypsin.

2. Preliminary periods of heating, up to 30 minutes in length, increased the degree of hydrolysis of egg white by trypsin. The extent of digestion of egg white by the successive action of pepsin and trypsin also varied directly with the length of the preliminary heating period to which the substrate was subjected.

3. These experimental results are interpreted as supporting the existence in raw egg white of an antitryptic agent which is slowly inactivated by heat.

BIBLIOGRAPHY

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