A STUDY OF THE HEAT STABILITY OF THE VITAMIN B FACTORS REQUIRED BY THE CHICK*

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Vitamin B₂ has been considered the more heat-stable constituent of the vitamin B complex since the early work of Goldberger, Wheeler, Lillie, and Rogers (1) in 1926. In the preceding paper (2) results were presented to show that vitamin B₂ was destroyed in a ration of natural foodstuffs when heated dry at 100° for 144 hours. These results show that under certain conditions vitamin B₂ can be inactivated at a temperature lower than that required for the destruction of vitamin B₁. It is evident, therefore, that further studies on the heat stability of the vitamin B fractions are necessary.

The present investigation deals mainly with results obtained when the diets described in the preceding paper and yeast were used for studying the stability of vitamins B₁ and B₂. However, the use of these rations also afforded us some opportunity to study the possible existence of the heat-labile vitamin B₂ which was first described by Williams and Waterman (3) as an additional vitamin B factor required by the pigeon and later regarded to be necessary for the chick by Eddy, Gurin, and Keresztesy (4).

Since the original ration used in these studies undoubtedly contained all additional heat-stable factors required by the chick, it was impossible to study such factors as vitamin B₅ described by Carter, Kinnersley, and Peters (5). However, we have studied the importance of additional factors for the chick when synthetic diets are used but this work will be reported in a later paper.

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Heat Stability of Vitamin B

Most of the work concerning the stability of vitamin B\textsubscript{1} has centered around the effect of pH on the rate of destruction. Some attempt has been made to correlate the action of pH and temperature. Sherman and Grose (6) showed that the decrease in acidity from pH 4.28 to pH 5.2 increased the rate of destruction to about the same extent as did the increase in temperature from 100\textdegree-110\textdegree.

The accepted method for destroying vitamin B\textsubscript{1} is autoclaving at 120\textdegree for 5 hours. Autoclaved yeast, which is free from or very low in vitamin B\textsubscript{1}, is used by practically all workers as a source of vitamin B\textsubscript{2}. There are at least two factors operative during autoclaving; namely, heat and moisture. Since the vitamin B\textsubscript{1} in a natural ration is destroyed completely when the ration is autoclaved at 120\textdegree for 5 hours, and destroyed only to a very slight extent when the ration is heated dry at 100\textdegree for 144 hours, the presence or absence of moisture must be a determining factor in inactivation.

Sherman and Spohn (7) observed some effect of moisture on the destruction of vitamin B\textsubscript{1} as early as 1923, before the importance of vitamin B\textsubscript{2} was recognized. They found no measurable diminution of vitamin B (B\textsubscript{1}) in milk powder when heated dry for 48 hours but found a destruction of about one-fourth of the total when fluid milk was heated at 100\textdegree for 6 hours. Sherman and Burton (8) suggested that the destruction of vitamin B is due to a hydrolysis or intramolecular rearrangement which is catalyzed by hydroxyl ions.

The following experiments were conducted to demonstrate the influence of moisture on the destruction of vitamin B\textsubscript{1}. Day-old white Leghorn chicks were placed in individual cages and fed Ration 241-A. The preparation of this ration is described in the preceding paper (2). Chicks grown on this ration invariably develop polyneuritis in 7 to 9 days. All the yeast preparations were made from one batch of fresh Red Star yeast. The yeast used for Groups 77 and 20 was dried at 100\textdegree for 24 hours. 4 per cent of the dry yeast was incorporated into the basal ration for Group 77 and 8 per cent was used for Group 20. Group 60 received 4 per cent yeast dried at 67\textdegree and then heated dry at 100\textdegree.
for 24 hours. Group 79 was given 4 per cent yeast dried at 67° and then heated moist at 100° for 24 hours. Group 80 was fed 4 per cent of yeast dried at 67° and then heated moist at pH 3.0 for 24 hours at 100°. Group 9, receiving the basal autoclaved ration alone, is included for comparison. The growth curves for four chicks in each group are given in Chart I.

It is readily seen that moisture is an essential factor in the destruction of vitamin B₁. The chicks receiving their supply of vitamin B₁ from yeast dried at 100° for 24 hours (Group 77) did not develop polyneuritis as early as those receiving the basal ration alone but all of them finally showed the characteristic deficiency symptoms. The addition of 8 per cent of this yeast protected the chicks from polyneuritis and produced normal growth (Group 20). This indicates that about one-half of the potency was destroyed during the heating process. Yeast dried at 100° loses its water in a few hours, but the presence of moisture even for this short time was sufficient to bring about a definite decrease in potency. These results demonstrate the importance of controlling the temperature when fresh yeast or moist foods are prepared for vitamin B₁ work.

When the yeast was dried first at 67° and then heated dry at 100° for 24 hours the chicks grew normally (Group 60), which shows that very little destruction takes place at 100° in the absence of moisture. The results for the chicks given yeast which had been dried at 67° and then moistened with distilled water before being heated at 100° for 24 hours are most striking. All the chicks developed severe polyneuritis when 7 to 9 days old (Group 79). The vitamin B₁ was destroyed completely under these conditions. The pH of the yeast mixture was approximately 6.4. The chicks which were fed yeast heated moist at pH 3.0 grew well and exhibited no signs of deficiency (Group 80). The rate of destruction is not only dependent upon the presence of moisture but also upon the hydrogen ion concentration of the solution. Guha and Drummond (9) found that the activity of a vitamin B₁ concentrate decreased about one-half when it was boiled at pH 5.0 for 24 hours.

Similar results were obtained when the basal ration (Ration 240) was used as the source of vitamin B₁ in place of the yeast. When 24 or 32 per cent of unheated ration or similar amounts of
the ration heated dry for 24 hours were added to Ration 241-A good growth was obtained, but when the ration was heated moist

for 24 hours before being incorporated into Ration 241-A poor growth was obtained both at the 24 and 32 per cent levels. Only

CHART I. Growth records of chicks showing the effect of heat, moisture, and pH on the destruction of vitamin B1 in yeast.
a few of the birds developed severe polyneuritis, which indicates that the destruction was not entirely complete. However, the decided difference in growth demonstrates that the presence or absence of moisture is also an important factor in the destruction of vitamin B₁ in a natural ration.

Since the vitamin B₁ in yeast and in a natural ration is destroyed completely by autoclaving and is destroyed to a large extent by heating moist at 100° for 24 hours, and since it is not destroyed when heated dry at 100° for 24 hours, it is logical to conclude that the presence of moisture is a more important factor in the destruction than is the increase in temperature. This conclusion substantiates the suggestion made by Sherman and Burton (8) that vitamin B₁ is destroyed by a hydrolysis in which the hydroxyl ions act as a catalyst.

**Heat Stability of Vitamin B₂**

There has been much interest during the past few years in the stability of vitamin B₂ to heat in alkaline media. Some of the workers claim that autoclaving in an alkaline medium destroys the vitamin, while others find no reduced potency under these conditions. Guha (10) has reviewed the work conducted on yeast and yeast extracts and has also shown that the vitamin B₂ in an aqueous solution of commercial liver extract is highly stable while the vitamin B₂ in an extract of fresh liver or fresh yeast is markedly unstable. He suggests that the difference is due to the presence or absence of some protective material in the commercial preparation.

Chick and Roscoe (11) studied the stability of vitamin B₂ at temperatures lower than 120°. They found that yeast heated for 2 hours at 90–100° in a faintly acid solution lost none of its potency, but when heated at pH 8.3 the activity was reduced one-half, and when allowed to remain in alkaline solution for 10 days at room temperature, one-third of the activity was lost.

The results given in the preceding paper clearly demonstrate that vitamin B₂ can be destroyed at temperatures lower than 120°. A few experiments were conducted to determine whether the inactivation of vitamin B₂ is due to heat per se or due to a reaction, the rate of which is increased by a higher temperature. Three portions of the basal ration (Ration 240) were heated at 100° for 24, 72, and
144 hours. Groups of four chicks, kept in individual cages, were fed each of these rations. The growth curves, including those for a group of chicks on the basal unheated ration, are given in Chart II. The chicks receiving the basal ration heated for 24 hours grew exceedingly well (Group 12). In fact, the rate of growth was slightly better than that obtained with chicks on the basal ration alone. The application of heat for a short time (100° for 24 hours), therefore, has no deleterious effect on vitamin $B_2$ but may actually improve the ration for growth. When the heating process is continued for 72 hours considerable destruction of vitamin $B_2$ takes place (Group 33) and when continued for 144 hours the destruction is complete or nearly so (Group 34).

Knowing that the ration could be rendered free of vitamin $B_2$ by proper heat treatment, we were interested in determining the...
effect of heat on a substance rich in vitamin B₂, such as yeast. It was first necessary to find approximately the amount of vitamin

\[ \text{Weight gain} \]

<table>
<thead>
<tr>
<th>Weight (g)</th>
<th>0</th>
<th>100</th>
<th>200</th>
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<tbody>
<tr>
<td>Age in Weeks</td>
<td>0</td>
<td>2</td>
<td>4</td>
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**CHART III.** Growth records of chicks showing the effect of heat treatment on the destruction of vitamin B₂ in yeast. P denotes the approximate time at which the symptoms of pellagra were first observed.

B₂ present in the yeast used, and this was done by feeding it at levels of 1, 2, and 4 per cent, added to Ration 240-H. It is evident from the growth curves in Chart III for Groups 94 and 95,
that 2 per cent of the yeast used contains sufficient vitamin $B_2$ for the chick, while 1 per cent is inadequate. When the air-dried yeast was heated at 100° for 144 hours and fed at the 2 per cent level (Group 97), the growth obtained was similar to that of chicks receiving 1 per cent of the untreated yeast. This is an approximate indication that 50 per cent of the vitamin $B_2$ was destroyed by the heat treatment. When the heated yeast was fed at the 4 per cent level, growth was somewhat improved, but there is still evidence of considerable destruction. When the yeast was first autoclaved and then heated for 144 hours at 100° (Group 99), there was slightly greater destruction than with the long heating alone.

Feeding trials have shown that 50 per cent of the basal Ration 240 is equivalent to 2 per cent of yeast in vitamin $B_2$ potency. Since yeast contains approximately 25 times more vitamin $B_2$ than the ration, it is reasonable that only one-half of the vitamin $B_2$ in the yeast should be destroyed under the conditions we have used. It is possible that with longer periods of heat treatment, a larger part of the vitamin may be destroyed.

The above results suggested that an oxidation process may take place during the heating which is responsible for the destruction. The results obtained from a few preliminary experiments indicate that this is not the case. Considerable destruction was observed when yeast was boiled in a water suspension for 144 hours and when heated in a tube filled with nitrogen. Further work is being continued in order to study more carefully the destruction under these conditions.

Does the Chick Require Vitamin $B_2$?

In 1930, Eddy, Gurin, and Keresztesy (4) concluded that chicks require the unstable vitamin $B_2$ for growth, even when fully supplied with vitamins $B_1$ and $B_6$ and with the other nutrients and vitamins. They found yeast, whole grains, and malt to be good sources of this factor, and that it is much more heat-labile than vitamin $B_1$. A temperature of 20° markedly reduced the amount of this factor in yeast if it was submitted to alkali treatment before drying.

During the past 2 years we have fed a number of rations, the constituents of which have been heated to temperatures of 100°
or above. We were greatly surprised to find that chickens made excellent growth on these rations because vitamin B_3 would certainly have been destroyed in our rations if it is as labile as Eddy and coworkers maintain.

The growth curves for chicks grown on three different rations are given in Chart IV. In each case all of the constituents of the ration except the cod liver oil and CaCO_3 were heated at 100° or above. Group 57 received Ration 240-H plus 6 per cent of autoclaved yeast. Ration 240-H is our basal Ration 240 heated at 100° for 144 hours and is deficient in vitamin B_3. The autoclaved yeast supplied this deficiency. Group 60 was given Ration

![Chart IV](http://www.jbc.org/DownloadedFrom)
241-A plus 4 per cent of yeast heated dry at 100° for 24 hours. Ration 241-A is our basal Ration 240 autoclaved plus 4 per cent of autoclaved yeast, and is deficient in vitamin B₁. The heated yeast supplied the lacking vitamin B₁. Group 64 also received Ration 241-A but in this case the vitamin B₁ was supplied by 32 per cent of Ration 240 heated dry at 100° for 24 hours.

The growth curves in Chart IV show that all of these rations produced normal growth or growth comparable in every way to the unheated ration. The results demonstrate without doubt that chicks do not require vitamin B₁, or any other factor which is destroyed by temperatures lower than 100°.

SUMMARY

1. Vitamin B₁ in yeast and in a natural ration was destroyed completely by autoclaving and inactivated to a large extent by heating moist at 100° for 24 hours, but was not reduced in potency when heated dry at 100° for 24 hours. The rate of destruction of vitamin B₁ in the presence of moisture was decreased with increase in hydrogen ion concentration.

2. The inactivation of vitamin B₂ in a natural ration heated dry at 100° was found to be dependent upon the length of time of heating. No noticeable destruction was observed after 24 hours of heating, but one-half of the potency was lost after 72 hours and the destruction was practically complete after 144 hours of heating. Dried bakers' yeast which contains about 25 times as much vitamin B₂ as the natural ration used lost one-half of its potency after heating dry for 144 hours.

3. Results are presented to show that chickens do not require the heat-labile vitamin B₂ or any other factor which is destroyed by heating at 100° or below for 24 hours.

BIBLIOGRAPHY

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