CCXI. THE HEAT-STABILITY OF VITAMIN B₂.

III. THE RATE OF DESTRUCTION AT VARIOUS REACTIONS OF VITAMIN B₂ CONTAINED IN DIFFERENT MATERIALS.

BY MARGARET HONORA ROSCOE¹.

From the Division of Nutrition, Lister Institute, London.

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WHILE little is known of the chemical and physical properties of vitamin B₂, it is generally recognised to be the most heat-stable member of the vitamin B complex. This fact made possible its original differentiation from vitamin B₁ and has been the basis of most subsequent methods of separation from other constituents of the vitamin B complex. For this reason much interest and work has been concentrated on ascertaining the degree of this heat-stability and the conditions governing it.

Williams and Waterman [1929] compared the effect on growth of rats of a given amount of brewer's yeast, given as source of vitamin B₂, before and after autoclaving for 6 hours at 120° at varying p_H , and showed that after this length of time destruction was slight in an acid medium but considerable at a slightly alkaline reaction (p_H 8.0) and practically complete at a strongly alkaline reaction (p_H 12-14).

Papers I and II of this series [Chick and Roscoe, 1930; Chick and Copping, 1930] contained the results of experiments on the heat-stability of vitamin B₂ as contained in dried yeast or in a watery yeast extract. The assays were carried out by means of rat growth tests and were checked by cures of dermatitis. It was shown that after heating at 120° for 4-5 hours in an acid medium (p_H 3.3-3.0) about half of this vitamin was destroyed, but after 4 hours in a slightly alkaline medium (p_H 8.3-7.1) nearly all was destroyed, while in a more alkaline medium (p_H 9.9-8.7) destruction was complete.

Halliday [1932] studied the heat-stability of vitamin B₂ present in protein-free milk, in quantitative experiments comparing the minimum amounts of each material needed to produce a given weight increase. Heating for 1 hour at 100° at p_H 4.3, 7.0 and 10.0 caused 10, 30 and 40 % loss, respectively, of vitamin B₂. Four hours' heating at the same reactions resulted in 30, 50 and 75 % loss respectively. It was therefore apparent that the rate of destruction by heat increased with increasing alkalinity and that after 4 hours at a temperature of 100° in a strongly alkaline medium there was great destruction of vitamin B₂.

In apparent contradiction of these results are those of Hassan and Drummond [1927], Reader [1929; 1930], Guha and Drummond [1929], Narayanan and Drummond [1930], Guha [1931, 1], who found that rats grew normally when receiving vitamin B₂ as small daily doses of marmite which had been heated at 110-125° for 1-3 hours in alkaline media, and who therefore concluded that vitamin B₂ was stable to heat and alkali. Only in two cases, however, was the marmite tested for its vitamin B₂ content before heating [Hassan and Drummond, 1927; Reader, 1929] and in no case was a determination made of the minimum doses of the material needed for normal growth before and after heating, so that the only conclusion to be drawn from this work is that vitamin B₂ was not entirely destroyed, in the materials tested, by 1-3 hours' heating in the alkaline medium employed.

¹ Beit Memorial Research Fellow.

The apparently divergent results obtained by different workers when investigating the heat-stability of vitamin B₂ as present in different materials have led to the supposition that variations in the source of the vitamin might affect the heat-stability. This factor was stressed by Guha [1931, 2]. He investigated the effect of heat (3 hours at 124–125°) on three different materials, (1) fresh ox-liver, (2) a watery solution of commercial liver extract (Eli Lilly, No. 343) and (3) a watery extract of brewer's yeast. In acid medium, no destruction of vitamin B₂ was observed in (2) or (3). But after heating in alkaline medium (initial p_H 9.0, final 7.6–6.1) the vitamin B₂ in (1) and (3) suffered 75 % and 90 % destruction respectively, while that in (2) appeared to be unaffected. These results together with previous ones were taken to show that the vitamin B₂ present in commercial liver extract (and in marmite, see Hassan and Drummond [1927]) was not readily destroyed by heat and alkali as was the case when present in fresh liver or in a watery yeast extract, the greater stability in liver extract and marmite being probably due to the presence of some protective agent.

In this paper therefore, experiments are reported, using various sources of vitamin B₂ similar to the above, *viz.*: a watery yeast extract, a watery liver extract and marmite. These have been heated at 120° for 1 hour in alkaline solution at approximately equal p_H values. In addition, further results are given of the effect of heating yeast extracts at acid and various alkaline reactions.

EXPERIMENTAL.

The method of testing for vitamin B₂ was that described by Chick and Roscoe [1928]. The basal diet and vitamin B₁ and B₄ supplement employed are described in an accompanying paper [Roscoe, 1933, 1]. The young rats weighing 35–45 g. were fed the basal diet only for 1 week during which time their weight was stationary and then received the vitamin B₁ and B₄ supplement and the doses of vitamin B₂. Growth was observed for 5 weeks, the amounts of each material providing sufficient vitamin B₂ to promote 50–60 g. increase in weight in this time being ascertained. This amount of growth was subnormal, and with more vitamin B₂ much better growth occurred; there was therefore no uncertainty, such as occurs when normal growth is the criterion, as to whether the doses administered were indeed the minimum amounts required to support this degree of growth. Four rats received each dose.

The following three materials were investigated.

(1) A watery extract of yeast, made from washed brewer's yeast with boiling 0.01 % acetic acid. 2 cc. of the extract were equivalent to 1 g. of the original yeast (dry wt.).

(2) Marmite, an autolysed yeast extract. This was dissolved in an equal weight of water before heating.

(3) Liver extract (Eli Lilly, No. 343), kindly supplied by the maker in the watery form prepared for intramuscular injection. 1 cc. of the extract was equivalent to 5 g. of the original whole liver.

These materials were all heated for 1 hour at 120° at approximately equal degrees of alkalinity (p_H 8.7–7.2). In addition, tests were made of the yeast extract (1) after treatment for 1 hour at three different degrees of alkalinity and for 5 hours in an acid medium. The heating took place in an autoclave at 15 lbs. pressure. Determinations of the p_H of the materials before and after treatment were carried out by means of a hydrogen electrode.

RESULTS.

The figures in Table I show that when watery yeast extract, marmite and liver extract were heated at 120° for 1 hour at p_H 8.7-7.2, 50 % destruction of the vitamin B_2 took place in each case. Thus there was no significant difference in the degrees of destruction by heat under these conditions of the vitamin B_2 contained in the three different materials.

Table I. *Destruction by heat of vitamin B_2 as contained in different materials.*

The following data are shown.

(a) The doses in g. of each material found necessary to promote standard growth (50-60 g. weight increase in 5 weeks), or, where this was not found, the nearest dose tested.

(b) The average weight increase in g. of the four rats receiving (a).

(c) The percentage destruction of vitamin B_2 , calculated from (a).

Material	Untreated		After heating 1 hour at 120°			p_H during heating
	(a)	(b)	(a)	(b)	(c)	
Yeast extract, R. X	0.25	(69)	0.5	(57)	50 % +	8.7-7.2
Marmite	0.15	(54)	0.3	(51)	50 %	8.6-8.5
Liver extract, Eli Lilly, No. 343	0.075	(49)	0.15	(48)	50 %	8.7-7.7

In Table II are shown the results of a more extensive set of experiments on the heat-stability of the vitamin B_2 in the yeast extract. The results of some previous work with similar extracts [Chick and Roscoe, 1930] are included for comparison.

Table II. *The effect of variation in the p_H of the substrate, and in time of heating, on the destruction of vitamin B_2 contained in a watery yeast extract.*

The following data are shown.

(a) The doses in g. of each material found necessary to promote standard growth (50-60 g. weight increase in 5 weeks), or, where this was not found, the nearest dose tested.

(b) The average weight increase in g. of the four rats receiving (a).

(c) The percentage destruction of vitamin B_2 , calculated from (a).

	Time during which material was heated at 120°								
Yeast ex- tract no.	Untreated		1 hour			4-5 hours			p_H during heating
	(a)	(b)	(a)	(b)	(c)	(a)	(b)	(c)	
R. X	0.25	(69)	—	—	—	0.25	(60)	0 % +	1.4
XII	0.20	(56)	—	—	—	0.4	(5 hours) (60)	50 %	3.3-3.0
XII	0.20	(56)	—	—	—	Trace undestroyed (4 hours)*			8.3-7.1
R. X	0.25	(69)	0.5	(57)	50 % +	—	—	—	8.7-7.2
R. VIII	0.25	(58)	1.5	(53)	83 %	—	—	—	9.0-8.7
R. XII	0.25	(78)	1.0	(60)	75 % +	—	—	—	9.3-8.5
XII	0.20	(56)	—	—	—	Complete destruction (4 hours)*			9.9-8.7

* Results obtained by Chick and Roscoe [1930].

As would be expected, the length of time for which heating was carried on had a considerable effect on the amount of destruction. Thus, while heating at 120° at p_H 8.7-7.2 for 1 hour destroyed only slightly more than 50 % of the vitamin, after 4 hours only traces remained undestroyed; in a more alkaline medium (p_H 9.3-8.5) after 1 hour rather more than 75 % destruction had occurred and after 4 hours (p_H 9.9-8.7) destruction was complete.

The rate of destruction was increased greatly with increasing p_H . While at p_H 1.4 an insignificant amount of the vitamin was destroyed even after 5 hours at 120°, at p_H 3.3–3.0 there was 50 % destruction after 4 hours; at a slightly alkaline reaction, p_H 8.7–7.2, the same percentage destruction occurred after 1 hour's heating, and at p_H 9.0–8.7 there was 83 % destruction after 1 hour.

Some of the above materials were also tested, before and after treatment, for their capacity to cure dermatitis [see Roscoe, 1933, 2]. The results were in good accordance with those obtained by the growth method.

DISCUSSION.

The results here obtained on the heat- and alkali-stability of vitamin B₂ in marmite and liver extracts are at variance with those of Hassan and Drummond [1927] and Guha [1931, 2], who found no appreciable destruction of vitamin B₂ in these materials after 1 or 3 hours' heating in an alkaline medium at 115°, as opposed to considerable destruction in the case of a watery yeast extract. In the present work the vitamin B₂ in marmite and in liver extract was as much affected as that in yeast extract.

The treatment here employed was similar to, but not identical with, that used by the above authors. The marmite was heated in a slightly, as opposed to a strongly alkaline medium [Hassan and Drummond, 1927]; the liver extract was heated at a similar reaction in both instances, but in this work for 1, as opposed to 3, hours [Guha, 1931, 2]. The treatment in the present work was thus in each case less severe, so that less, rather than more, destruction was to have been expected. Hassan and Drummond, however, did not determine the minimum amounts of the untreated marmite needed for normal growth, so that their results, while showing that adequate vitamin B₂ to support growth had survived the treatment, did not prove that some degree of destruction had not occurred. The data in Guha's paper are not given in sufficient detail to show whether this criticism may not be applied to his experiments also.

The results reported in this paper emphasise the fact that the destruction of vitamin B₂ by heat should be regarded as a time-process. As a result of observations on heating for 1 hour [Hassan and Drummond, 1927; Reader, 1929], the vitamin was judged to be very heat-stable in an alkaline medium, whereas Chick and Roscoe [1930], from experiments in which heating was carried out for 5 hours, considered that the vitamin was heat-labile in an alkaline medium. To a certain extent this discrepancy is explained when the different durations of heating are taken into account.

SUMMARY.

1. No difference was found in the stability to heat and weak alkali of vitamin B₂ as contained in (a) a watery yeast extract, (b) marmite solution or (c) a watery liver extract (Eli Lilly, No. 343). When heated for 1 hour at 120° at p_H 8.7–7.2 all three materials lost 50 % of their original vitamin B₂ potency. Thus no support was obtained for the theory that the resistance of vitamin B₂ to heat and alkali varies according to the material in which it is present.

2. In confirmation of previous work vitamin B₂ was found to be relatively heat-stable in acid solution and the rate of destruction to be increased rapidly with increasing alkalinity.

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