# STABILITY OF THIAMINE TO HEAT

## I. EFFECT OF pH AND BUFFER SALTS IN AQUEOUS SOLUTIONS*

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It has been known for some time that thiamine is less stable to heat in alkaline solution than in acid solution ((1) p. 104, (2)). Solutions of thiamine at pH 3 and 6 are said to be unaffected on heating for 30 minutes at 100° (2). They are reported to be unaffected by sterilizing at pH 3.5 for 1 hour at 100°, or 20 minutes at 120° (2). Sherman and Burton (3) found that tomato juice lost 20 per cent of its thiamine content during 4 hours heating at 100° at the natural pH of 4.28, but when the pH was raised to 9.2, the destruction was 60 to 70 per cent in 1 hour. Keenan, Kline, Elvehjem, and Hart (4) showed that vitamin $B_1$ in yeast, liver, and in a natural grain ration was inactivated completely by autoclaving for 5 hours. Eddy, Kohman, and Carlsson (5) stated that no cooking or canning process affects appreciably the content of vitamins A and B in green peas, while the Medical Research Council stated in a special report (6) that canned foods of all descriptions may contain very little or no vitamin $B_1$.

It is apparent from the above statements that there are factors involved in the destruction of thiamine by heat which are little understood, since losses of thiamine may occur in one food but not in another, during similar processing, and since different workers draw different conclusions from experimental data on the effects of processing of foods. As an aid to the more complete understanding of the problem it is of interest to make a systematic study of the behavior of pure thiamine at various pH values in different buffer solutions. Such a study should indicate whether or not the destruction of pure thiamine due to heat is a factor of pH only, or whether the buffer system itself is of importance in determining the amount of destruction. The purpose of this paper is to present the results of such a study as were obtained in a series of experiments with pure thiamine.

### EXPERIMENTAL

The pH values of the solutions were determined by means of a Leeds and Northrup type 7661-A1 instrument, with which it is easily possible to

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reproduce values to within 0.05 pH unit. All solutions were subjected to pH determination both before and after treatment.

The thiamine assays were made by the thiochrome procedure for estimation of thiamine, as outlined by the Research Corporation Committee (7) and by spectrophotometric examination of the ultraviolet absorption spectra. The spectrophotometric studies were conducted by means of a model DU Beckman photoelectric spectrophotometer, having quartz optics, with a hydrogen discharge tube as the source of continuous radiation in the ultraviolet.

The absorption spectrum of thiamine is known to be affected by the pH of the medium ((1) p. 103, (8–14)). It was found, however, that the ab-

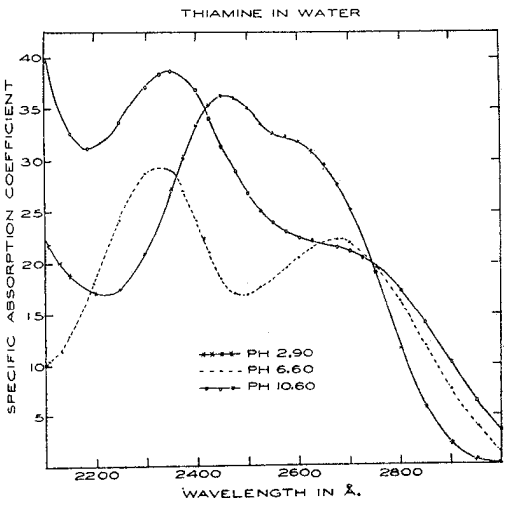


FIG. 1

sorption curve is so radically affected by small changes in pH that duplication of data was not possible unless the pH values were controlled very closely, to about 0.05 pH unit. Fig. 1 shows typical absorption data obtained for aqueous solutions of pure thiamine chloride hydrochloride as supplied by Merck and Company, Inc. (in the remainder of this paper the term "thiamine" refers to thiamine chloride hydrochloride). The specific absorption coefficients were calculated from the Lambert-Beer equation (15), $\log I_0/I = \alpha cl$, where $I_0$ = the intensity of radiation transmitted by the solvent, $I$ = the intensity of radiation transmitted by the solution, $\alpha$ = the specific absorption coefficient, $c$ = the concentration in gm. per liter, and $l$ = the length of solution in cm.

At the wave-length 2600 Å. (an analytical point selected by the authors for analysis of binary systems in another study), the relation between the

specific absorption coefficient and the pH of the aqueous solution was studied. Fig. 2 shows this relationship graphically, between pH 1 and 11. Through the use of this curve, the specific absorption coefficient may be readily learned for the pH values of the solutions being examined. It may be seen in Fig. 2 that the absorption coefficient at this wave-length decreases rapidly as the pH rises from 3 to 5.5, remains fairly constant between pH 5.5 and 7.5, then increases rapidly as the pH rises from 7.5 to 10.

The solutions of thiamine used throughout the series of studies were prepared by the addition of 10 ml. portions of a stock solution, containing 100 $\gamma$ of pure thiamine per ml., to 100 ml. volumetric flasks. The flasks were then filled almost to the mark with water (or the aqueous buffer

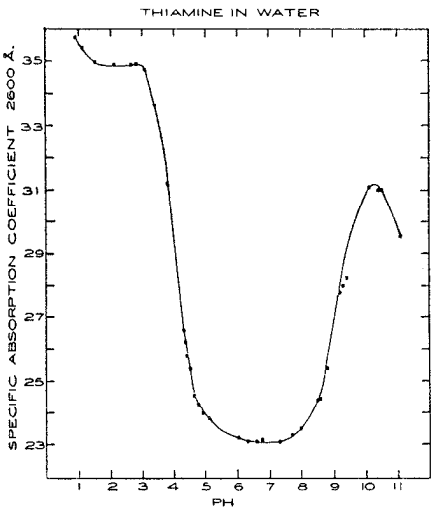


FIG. 2

solution), final adjustment of pH made, and the flasks filled to the mark. This procedure yielded solutions containing 10 $\gamma$ of thiamine per ml., a concentration well within the range of that reported in many meats and other foodstuffs. Aliquots of these solutions were then placed in smaller flasks and immersed in boiling water for the desired time, after which they were cooled rapidly to room temperature. Spectrophotometric examinations and chemical analyses were then made on aliquots of the solutions both before and after the heating period.

The unbuffered solutions were brought to the desired pH by the addition of 0.01 N sodium hydroxide or 0.01 N hydrochloric acid, as required. At extreme pH values more concentrated reagents were required. The pH of thiamine in distilled water at a concentration of 10 $\gamma$ per ml. is about 4.25.

The buffer solutions were prepared according to the so called standard

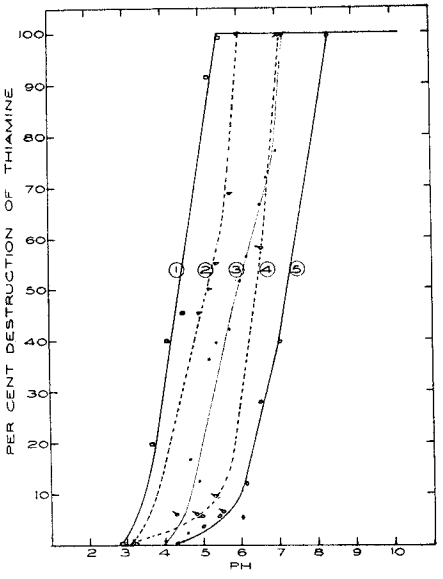


FIG. 3

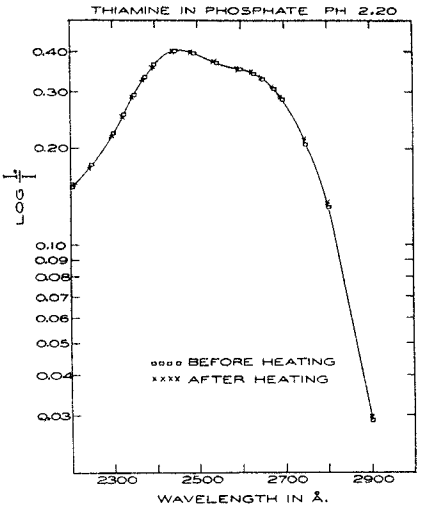


FIG. 4

FIG. 3. Effect of heat on thiamine in solution. Curve 1 represents borate buffer; Curve 2, unbuffered solution after heating for 60 minutes; Curve 3, unbuffered solution after heating for 30 minutes; Curve 4, acetate buffer; Curve 5, phosphate buffer.

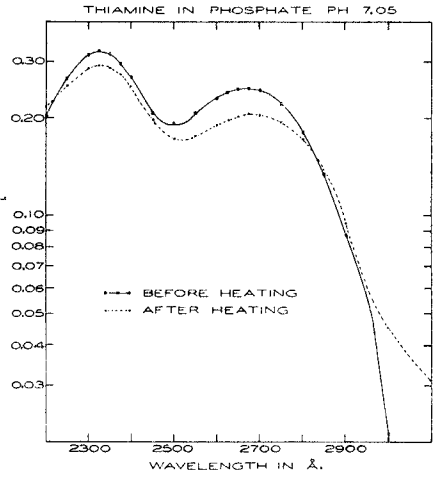


FIG. 5

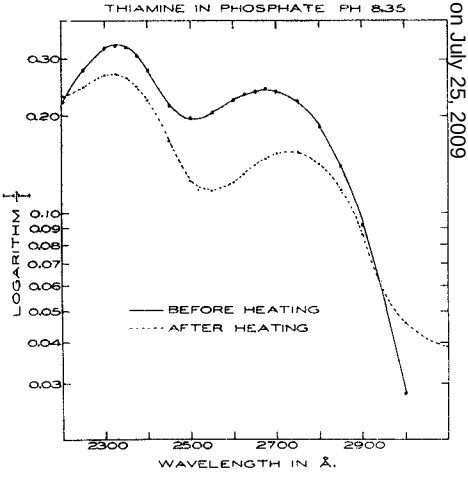


FIG. 6

methods (16, 17). The acetate solutions were those of Walpole (17), the borate solutions were those of Palitzsch (17), and the phosphate solutions those of Sørensen (16). At pH values outside the ranges covered by the

buffer systems, sodium hydroxide or hydrochloric acid was added to obtain the desired pH in the presence of the buffer salts.

Fig. 3 is a graphic portrayal of the results obtained in unbuffered solution after heating in boiling water for periods of 30 minutes and 60 minutes, respectively, and in the buffer solutions after a heating period of 60 minutes.

Figs. 4, 5, and 6 present typical spectrophotometric data obtained on the solutions before and after the heating periods. While the curves in these figures were obtained on phosphate solutions, the same results were obtained in the unbuffered series and in the borate series. Lack of space does not justify presenting all of them. It was not practical to attempt spectrophotometric interpretation of the acetate solutions owing to strong absorption by the acetate ion at wave-lengths shorter than 2500 Å. All the absorption spectra except those of Fig. 1 are plotted with the logarithm of log $I_0/I$ as the ordinate, and wave-length in Å. as the abscissa, for the purpose of ease in comparison. Changes in concentration result merely in moving the curve to a different height on the ordinate, without altering its shape (18). Absorption measurements were reproducible within about 1 per cent.

## DISCUSSION

The data presented in this report represent nearly 200 thiamine analyses. While an occasional analysis failed to fall exactly on the curves shown in Fig. 3, such instances were rare and are believed by the authors to be due to inherent errors in the thiochrome method or the technique of the operator. It was estimated as the result of analyses on known solutions that individual results may vary about 5 per cent from the known, or a total of about 10 per cent between the extremes. As is evidenced by Fig. 3, all of the curves are so steep that the pH effect in each instance is far greater than this, even within rather narrow limits. The destruction of thiamine rises from 0 to 100 per cent within 2 or 3 pH units.

The effect of variations in the electrolyte system at a given pH on the stability of thiamine to heat is striking. For example, at pH 5.4, as shown in Fig. 3, there was 100 per cent destruction of the vitamin during 1 hour's heating in the presence of borates, 60 per cent destruction in unbuffered aqueous solution, 10 per cent in the presence of acetates, and about 3 per cent in the phosphate solution. At pH 7, there was 100 per cent destruction in the unbuffered solution and in the borate and acetate solutions, while only 40 per cent of the thiamine was destroyed under similar conditions in the phosphate solution. The effect of time on destruction of thiamine in unbuffered solution is illustrated by the figures at pH 6.0. All the vitamin was destroyed during 1 hour's heating, while about 50 per cent was destroyed in the 30 minute period. When these samples

were allowed to stand for as long as 10 days in the refrigerator (5°) without going through the heating period, no losses were observed either in the unbuffered or the buffered solutions.

It can hardly be concluded from the above that the destruction is simply a salt effect, for in this instance the amount of destruction should have been the least in the unbuffered solutions, where the least amount of salt was present.

A quantitative comparison of the spectrophotometric data on thiamine presented in Fig. 1 with the values in the literature cannot be made in most instances, owing to difficulty in interpolating the values published graphically and also to lack of sufficient data regarding the pH and solvent.

Melnick (14) has published molecular extinction coefficients for thiamine at pH 7.4 in phosphate buffer solution, using a Bausch and Lomb medium spectrograph. His values were 10,250 and 6000 at the maxima 2350 and 2650 Å., respectively. The values obtained in this laboratory for the thiamine used in these studies with the photoelectric spectrophotometer were 10,843 and 8383 at the above wave-lengths, at pH 7.4, in Sørensen's phosphate solution. Values obtained on a sample of U.S.P. reference standard thiamine were 10,885 and 8290 under the same conditions.

There is considerable evidence that chemical methods of assay for thiamine are not completely reliable ((1) p. 128–130); therefore, one may well suspect that the ions present in the various buffer series encountered in these experiments may have interfered with the thiochrome procedure, thereby vitiating the conclusions regarding the losses in the various systems. The spectrophotometric examinations typified in Figs. 4, 5, and 6 were made as a means of establishing whether or not destruction of thiamine actually occurred when indicated by the thiochrome assay. No instances were found in which the spectrophotometric data were contrary to those obtained by the thiochrome method. When no destruction of thiamine was indicated by the thiochrome method, the absorption curves from solutions before and after heating were perfectly superposable, matching at all points, as shown in Fig. 4. When losses were found by the thiochrome method, the absorption curves indicated the destruction by shifts in the curve both as to height and points of maxima, as shown in Figs. 5 and 6. The greatest changes in the absorption spectra were found in those solutions in which destruction was greatest, as determined by the thiochrome procedure. This indicates that under the conditions encountered the thiochrome method was reliable as an index of the thiamine present. No bioassays were made.

It is interesting to note that in all instances in which complete destruction of thiamine occurred the resulting solutions yielded characteristic absorption spectra, as shown in Fig. 6. The fact that these spectra closely resemble

the spectra of compounds of the pyrimidine type similar to the pyrimidine nucleus of thiamine leads one to the conclusion that the destruction in all the instances investigated did not break down the pyrimidine component of the molecule. It has been stated that sulfites cause cleavage of the thiamine molecule (19, 20), also that barium nitrite and possibly sodium acetate cause this same cleavage (21, 22). The authors found the absorption characteristics mentioned above in all the buffers examined, as well as in the unbuffered solution to which no salts had been added but in which complete destruction of the thiamine was indicated. While it must be remembered that the absorption curve shown in Fig. 6 is probably complicated by the thiazole residues, its similarity to the pyrimidine curves of Uber and Ver-

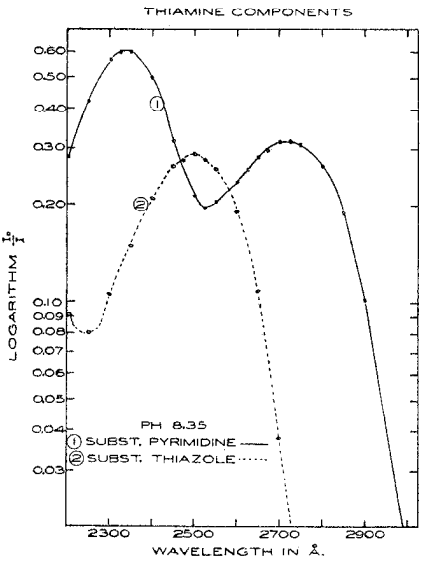


FIG. 7

brugge (13) is interesting. Through the courtesy of Dr. Randolph T. Major of Merck and Company, Inc., the authors obtained samples of the substituted pyrimidine and thiazole compounds from which thiamine is synthesized. The absorption spectrum of the pyrimidine component in phosphate buffer at pH 8.3 is shown in Fig. 7, together with the spectrum of the thiazole. The similarity of the pyrimidine curve to the lower curve in Fig. 6 is apparent. The sulfite cleavage products have been shown to possess no vitamin $B_1$ activity (19, 20). But more recent studies (23, 24) indicate that synthesis of thiamine from the cleavage products may occur in the digestive tract.

The data presented here, while not complete in the sense of covering all

natural buffers, indicate the need for caution in making generalizations in regard to the stability of thiamine under various conditions of processing. The varying results reported by workers on different foods may quite possibly be due not only to variations in pH within the tissues, but also to variations in the electrolyte systems and possibly to other factors such as the protein systems involved. It has been noted that thiamine is more stable in biological tissues than in pure solution (25). Williams (21) has suggested that possibly cocarboxylase (the pyrophosphoric acid ester of thiamine) may exhibit a stability to heat different from that of pure thiamine. Greenwood, Beadle, and Kraybill (26) have found that certain proteins exert a strong protecting action on thiamine, and that cocarboxylase is only slightly more stable to heat than is thiamine.

## SUMMARY

Results of chemical and spectrophotometric examination of nearly 200 solutions of pure thiamine indicate that the stability of thiamine to heat is a function not only of pH but also of the electrolyte system involved. At pH 5.4, during 1 hour's heating in boiling water there was 100 per cent destruction of the thiamine in the presence of borates, 57 per cent destruction in unbuffered aqueous solution, 10 per cent destruction in the presence of acetates, and 3 per cent in phosphate solution. In each type of solution, destruction rose from 0 to 100 per cent within the range of 2 to 3 pH units during 1 hour's heating period.

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