HYDROLYTIC DESTRUCTION OF THIAMINE, ESPECIALLY IN THE PRESENCE OF CYANOCOBALAMIN

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The hydrolytic destruction of thiamine hydrochloride in various buffer solutions at pH 4·5 at 100° has been investigated. The rate of hydrolysis approximated to that of a first order reaction, the salts of weak organic acids acting as catalysts so that buffer salts could not retard the decomposition. It was confirmed that the hydrolytic products of thiamine affect the stability of cyanocobalamin, and that low concentrations of ferric ions can protect cyanocobalamin against the effects of thiamine breakdown products, but this occurs without appreciably affecting the stability of thiamine itself.

THE formulation of stable solutions containing both thiamine hydrochloride and cyanocobalamin, possibly with other components of the vitamin B complex, presents a number of problems. Thiamine hydrochloride is stated by the British Pharmacopoeia to be stable in solutions at pH values below 5 but to deteriorate rapidly in neutral or alkaline solutions, especially on contact with air. The dependence of stability on pH has been demonstrated by Farrer (1941) and Beadle, Greenwood and Kraybill (1943). Booth (1943) and Farrer (1945) showed that the stability of the vitamin in solution was affected by certain buffer salts; and the same authors (Booth, 1943; Farrer, 1947) found that some metal ions at trace concentrations also influenced stability. The rate of decomposition of thiamine has also been held to be dependent on its initial concentration (McIntire and Frost, 1944).

In solutions containing thiamine, cyanocobalamin, and other vitamins of the B complex, rapid destruction of cyanocobalamin occurs and has been ascribed to the effects of nicotinamide and the products of hydrolytic breakdown of thiamine (Blitz, Eigen and Gunsberg, 1954; Feller and Macek, 1955). Mukherjee and Sen (1957, 1959) reported that cyanocobalamin could be protected against this destruction by ferric chloride in low concentrations; similar protection by ferric salts had been reported by Skeggs (1952) and Newmark (1958).

It was thought that the stability of cyanocobalamin in the presence of thiamine might be improved if the hydrolytic breakdown of the thiamine could be retarded by the use of a suitable buffer salt at optimal concentration, and that stability might be also increased by ferric ions in low concentration. We undertook to determine whether such stabilisation was possible and to provide some information on the mechanisms of the reactions involved in the decomposition of the vitamins.

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In most of the previous investigations with thiamine alone, concentrations of thiamine were lower than in many vitamin preparations. The studies were often extended over a wide range of pH, and decomposition from boiling or exposure to higher temperatures was often determined. The conditions we selected were: one pH value of 4·5; the use of therapeutic concentrations of 10 mg. of thiamine hydrochloride per ml. and 100 μg. of cyanocobalamin per ml.; the use of acetate, succinate, glutamate and tartrate buffers, which were considered sufficiently non-toxic for use in either oral or parenteral preparations, at a concentration of 0·15M; exposure of the solutions to a temperature of 100° for fixed periods.

First, the effect of different anions on the rate of hydrolytic breakdown of thiamine alone was investigated. Then the effects of anion concentration and ferric chloride on the rates of destruction of both thiamine and cyanocobalamin were studied. The results were considered in terms of first order reaction kinetics and the specific reaction rates (velocity constants) obtained were compared with those calculated from previously reported values.

Experimental

Analytical reagent grade chemicals were used except thiamine hydrochloride and cyanocobalamin, which were of the British Pharmacopoeia, 1958. Water was freshly distilled and free from carbon dioxide. The solutions were filled into ampoules, which were sealed and heated in a boiling water bath for 1, 2 and 4 hr., after which the ampoules were rapidly cooled. Thiamine was determined gravimetrically by precipitation with silicotungstic acid; cyanocobalamin was determined microbiologically by the *Escherichia coli* cup-plate assay of Robinson, Fitzgerald and Grimshaw (1956), the assessment of potency and validity of the assay being as described by these authors.

In the experiments in which ferric chloride was added to the vitamin solution, succinate and acetate buffer solutions could not be used because they precipitated iron. The glutamate buffer could be used in the experiments only at the lower concentration because of the limited solubility of glutamic acid.

Results and Discussion

The destruction of thiamine in the four 0·15M buffer solutions, and also in 0·15M and 0·30M tartrate buffer solutions in the presence of cyanocobalamin, is shown in Table I, together with the pH determined before and after the longest heat treatment and the calculated specific reaction rates for the hydrolytic reaction. Determinations of cyanocobalamin remaining in those solutions containing both vitamins are listed in Table II, together with the calculated specific reaction rates for the decomposition of cyanocobalamin. The specific reaction rates were calculated by the standard graphical method, which also verified that the destruction of both vitamins approximated to reactions of first order.

In all solutions, including the unbuffered control solutions, the pH shift on heating was small and often was negligible.

Destruction of Thiamine

It is well known that thiamine hydrochloride is destroyed on heating by hydrolytic cleavage to yield the pyrimidine and thiazole moieties (Rosenberg, 1942). Since the hydrolysis of thiamine is a first order reaction, the buffer salt emerging unchanged at the end of the reaction, a

TABLE I

DESTRUCTION OF THIAMINE AT 100° IN SOLUTIONS CONTAINING 10 MG. THIAMINE HYDROCHLORIDE PER ML. WITH OR WITHOUT ADDITION OF 100 μG. CYANOCOBALAMIN PER ML. AND 250 P.P.M. FERRIC CHLORIDE

Vehicle	Additions	Thiamine hydrochloride per cent remaining after heating at 100° — Time in hr. 1	2	4	pH shift Initial	Final	k_1 thiamine
No buffer ..	Thiamine	96·3	95·3	91·0	4·45	4·30	$4·09 \times 10^{-4}$
0·15M Acetate ..	,,	94·0	89·7	79·0	4·50	4·42	$9·8 \times 10^{-4}$
0·15M Tartrate ..	,,	93·0	89·3	78·5	4·56	4·44	$1·01 \times 10^{-3}$
0·15M Succinate	,,	92·8	88·9	78·9	4·35	4·32	$1·00 \times 10^{-3}$
0·15M Glutamate	,,	93·5	88·1	78·8	4·50	4·40	$1·02 \times 10^{-3}$
No buffer ..	Thiamine, cyanocobalamin	97·2	96·3	89·9	4·50	4·30	$4·22 \times 10^{-4}$
0·15M Tartrate ..	,, ,, +Fe	93·2	87·8	76·1	4·50	4·40	$1·08 \times 10^{-3}$
0·3M Tartrate ..	,, ,,	90·0	86·8	75·2	4·56	4·42	$1·29 \times 10^{-3}$
0·3M Tartrate ..	,, ,, +Fe	92·6	85·0	73·8	4·54	4·50	$1·25 \times 10^{-3}$

general acid-base catalysis is suggested. At the pH of the solutions used, concentrations of hydrogen and hydroxyl ions are small, so that any increase in the reaction rate is dependent on the catalytic coefficients of the undissociated acid and the basic anion, and on the concentration of the anion. Since catalytic coefficients of acids are related to their dissociation constants, a similar contribution to catalytic power would be

TABLE II

CYANOCOBALAMIN DESTRUCTION AT 100° IN SOLUTIONS AT PH 4·5 CONTAINING 10 MG. THIAMINE HYDROCHLORIDE PER ML. AND 100 μG. CYANOCOBALAMIN PER ML. WITH OR WITHOUT THE ADDITION OF 250 P.P.M. FERRIC CHLORIDE

Vehicle	Additions	Cyanocobalamin, per cent remaining after heating at 100° — Time in hr. 1	2	4	k_1 cyanocobalamin
No buffer	Thiamine, cyanocobalamin	93·2	64·4	36·7	$3·62 \times 10^{-3}$
0·15M Tartrate ..	,, ,, +Fe	77·2	52·8	46·0	$3·45 \times 10^{-3}$
0·3M Tartrate ..	,, ,,	72·0	47·5	27·7	$5·67 \times 10^{-3}$
0·3M Tartrate ..	,, ,, +Fe	89·6	86·0	68·4	$1·60 \times 10^{-3}$

expected from the use of acids with approximately equal dissociation constants. This was found (Table I); similar total specific reaction rates were calculated among the four buffer solutions derived from weak acids of pKa values between 4 and 5. Increase in the anion concentration in the two tartrate solutions led to an appreciable increase in the specific reaction rate.

Specific reaction rates for the destruction of thiamine were calculated from the results of Farrer (1945), which relate to the use of phosphate-citrate and borate-succinate buffers where the reaction was of first order, and Booth (1943); here the rates of hydrolytic destruction in phthalate and phosphate-phthalate buffers were found to approximate to a second order reaction. The data reported by Beadle (1943) and Mukherjee and Sen (1959) were not sufficient for such calculations.

Beadle (1943) found that the nature of the buffer salt used in solutions of thiamine affected the rate of destruction, both acetate and phosphate retarding the rate considerably compared with other salts. We found little evidence of such retardation by acetate. Another difference between our results and those previously reported is in the effect of metals on the rate of decomposition of thiamine. Farrer (1947) reported that various metals at low or trace concentrations affected the rate of hydrolysis, which was occasionally retarded but more usually accelerated. We found ferric chloride, 250 p.p.m., in tartrate buffer solutions not to affect the rate of thiamine destruction significantly.

Farrer (1948), using 5–100 μg. of thiamine per ml. in phosphate-citrate buffer (0·2M/0·1M) at pH 4·5 and heating at 100°, concluded that the rate of decomposition of thiamine was dependent on its initial concentration. His results may be explained by the fact that the lower the thiamine concentration, the higher would be the relative concentration of buffer anion, with resultant increase in the rate of reaction.

Destruction of Cyanocobalamin

Destruction of cyanocobalamin when heated in buffered, weakly acid solution is accelerated by reducing agents. Macek (1961) explained this in terms of oxidation-reduction reactions of cobalt co-ordination complexes. Among agents capable of reducing cyanocobalamin in multivitamin preparations, the best known is ascorbic acid, but the thiazole moiety resulting from hydrolytic cleavage of thiamine exerts a similar effect (Blitz, Eigen and Gunsberg, 1954). This was confirmed by Mukherjee and Sen (1957, 1959) who also showed that, in this instance, low concentrations of ferric chloride could protect cyanocobalamin. Skeggs (1952) had previously shown that the stability of cyanocobalamin solutions was appreciably improved by saccharated iron oxide, and later Newmark (1958) demonstrated that cyanocobalamin, in the presence of ascorbic acid and other constituents of multivitamin solutions, could be successfully stabilised by a number of soluble iron salts, some of which were of sufficiently low toxicity to include in pharmaceutical preparations.

Gambier and Rahn (1958) concluded that if the ratio of thiamine to cyanocobalamin in solutions was less than 120 to 1, decomposition of cyanocobalamin was minimal, provided a critical pH was maintained and heat was avoided in the preparation of injection solutions.

Our results confirm that ferric chloride, 250 p.p.m., added to a solution of thiamine and cyanocobalamin in a tartrate buffer protects the cyanocobalamin from destruction, without affecting the decomposition of thiamine. It can be seen from Table II that the tartrate buffer enhanced

the destruction of cyanocobalamin, compared with the unbuffered solution, an effect explained not by an action of tartrate on cyanocobalamin stability, but by a greater concentration of thiamine hydrolytic products caused by the tartrate. The decomposition of cyanocobalamin approximated to a first order reaction. The reaction between the decomposition products of thiamine (probably the thiazole moiety) and cyanocobalamin was effectively retarded by ferric ions, which implies that the decomposition involves a reduction reaction as postulated by Mukherjee and Sen (1957, 1959) and Macek (1961).

Thus the rate of hydrolytic decomposition of thiamine by the addition of buffer salts at pH 4·5 could not be retarded. Conversely, these buffer salts accelerated the decomposition of thiamine, an effect attributed to both the undissociated acid and its anion and presumably dependent on their catalytic coefficients and on the anion concentration. This is of little practical significance with solutions of thiamine alone, which are adjusted to a more acid reaction to give better stability, but it is significant in multivitamin liquid preparations that are frequently adjusted to a pH of between 4 and 5.

The nature of the buffer salts examined did not appear to influence the stability of thiamine hydrochloride solutions to boiling and presumably would similarly fail to affect stability during prolonged storage at more moderate temperatures. This behaviour may be found when acids of similar dissociation constants and hence similar catalytic coefficients are employed as buffering agents.

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