CLXXIII. A STUDY OF THE DESTRUCTION OF VITAMIN B₁ IN BLOOD

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IN a quantitative study of the excretion of vitamin B₁ in human urine [Harris & Leong, 1936], it was observed that, with the subjects studied, the daily urinary output of the vitamin amounted to only about 10 % of the daily intake. Later, in a more detailed study on rats [Leong, 1937], evidence was obtained that a certain amount of the ingested vitamin could not be accounted for in the total urinary and faecal excretions and that this difference between intake and output could not be ascribed entirely to storage in the tissues. Thus it was clear that some vitamin B₁ had disappeared in the body, although the mechanism of (and the factors contributing to) this disappearance remained obscure. This disappearance of the vitamin may be due (among other causes) to (1) conversion into inactive products during metabolism in the tissues and in the blood, and (2) destruction in the alimentary tract, which includes the possible bacterial decomposition of the vitamin in the lower intestine. It was felt that a study of the stability (or the possible destruction) of vitamin B₁ in blood at body temperature might yield useful information bearing on these points.

The effect of heat on the antineuritic vitamin seems to depend mainly on (1) the pH and (2) the amount of moisture present. Its stability to dry heat has been shown by numerous workers, among whom may be mentioned the following: Holst [1907], Nitzescu [1923], Sherman & Spohn [1923] and Keenan *et al.* [1935]. The destruction of vitamin B₁ when heated under various conditions in contact with moisture has also been widely reported [Grijns, 1901; Sherman & Burton, 1926; Rosedale & Oliveiro, 1928; Elvehjem *et al.* 1932; Ohdake & Yamagishi, 1935]. No report appears to have been published, however, on the stability of crystalline vitamin B₁ at 37°.

EXPERIMENTAL

Stock solution of vitamin B₁. A solution of the vitamin was prepared by dissolving crystalline vitamin B₁-HCl in distilled water so that 1 ml. contained approximately 2 I.U. (The pH of this solution was about 6.) This particular concentration was chosen because 1 ml. of this solution represented a convenient dose for assay by the bradycardia method. For details of the method see Drury & Harris [1930], Birch & Harris [1934] and Leong & Harris [1937]. This stock solution was kept in an evacuated flask at 0° during the course of the experiment.

Stability of vitamin B₁ at 0°. In order to demonstrate the stability of vitamin B₁-HCl when kept at 0° in simple aqueous solution, a series of assays (which were carried out simultaneously with the tests described below) were done on 1 ml. samples of the stock solution. In Table I are arranged the results of these tests in order of the dates on which they were done. It will be seen that the individual variations of the results are within the range of the experimental error associated with the bradycardia tests [cf. Leong & Harris, 1937], and that vitamin B₁ is stable in aqueous solution at 0° for at least 3 months.

Table I. *Stability of vitamin B₁ in aqueous solution at 0°.*

Date of test	Cure of bradycardia in days (individual animals)
4. i. 37	4.5, 6
20. ii. 37	3
24. ii. 37	5.5
27. ii. 37	4, 5
3. iii. 37	4.5
15. iii. 37	7
29. iii. 37	4, 4, 6
3. iv. 37	5, 6
Average of 13 tests	4.9

Destruction of vitamin B₁ in blood at 37°. The details of the experimental technique were as follows. The blood was obtained from normal rats which had been previously starved for 6 hr. (Control tests with blood drawn under such conditions have shown that it contained insignificant amounts of vitamin B₁.) The blood was drawn by heart puncture and precautions as to sterility were observed throughout.¹ The pH of the blood plasma was about 7.3. The stock solution of vitamin B₁ was sterilized by filtration through a Seitz filter immediately before use and 1 ml. of this solution was incubated with 1 ml. of the oxalated blood at 37° for 24 hr., after which the mixture was assayed for vitamin B₁ by the bradycardia method. For purposes of comparison, tests were also carried out on a mixture of 1 ml. of the solution of vitamin B₁ and 1 ml. of freshly drawn blood. All the test doses were promptly consumed by the animals.

The results, giving the duration of cure of bradycardia in days, are shown in Table II. It will be seen that this heat treatment of vitamin B₁ in blood resulted in a certain amount of inactivation of the vitamin.

Table II. *Destruction of vitamin B₁ in blood heated at 37° for 24 hr.*

1 ml. vitamin B ₁ solution + 1 ml. blood		
	Before heat treatment Cure of bradycardia (days)	After heat treatment Cure of bradycardia (days)
	2	2
	3	2
	3	2.5
	4	3
	4	3
	4.5	4
	4.5	4
	5	4
	5	4.5
	5	5
	5.5	.
	6	.
	6	.
	6.5	.
	7	.
Mean	4.74 ± 0.49	3.40 ± 0.32
Standard deviation	1.38	1.04
Difference of means	1.34	
Standard error of difference of means	0.49	

¹ I wish to thank Dr A. A. Salam for help in bacteriological technique.

A statistical analysis of the results bears out this conclusion provided that it can be assumed (as seems justified) that statistical methods can be applied to samples of this size. For each series of results the standard deviation (σ), where $\sigma = \sqrt{\frac{\sum d^2}{n-1}}$, has been calculated (see Table II which also gives the standard error of the mean (ϵ) where $\epsilon = \frac{\sigma}{\sqrt{n}}$). The standard deviation of the response of an individual rat in these tests is of the same order as that previously observed when larger numbers of assays (upwards of 270) were carried out with the International Standard acid clay [Leong & Harris, 1937]. The standard error of the difference between the two means is therefore $\sqrt{\left(\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}\right)} = 0.49$. The difference between the two averages is 1.34. This is about three times its standard error, which suggests that the observed difference is unlikely to have occurred by chance.

Incubation of vitamin B₁ with boiled blood. Having shown that there has been a destruction of vitamin B₁ when the latter was incubated with rat's blood at 37°, it became of interest to ascertain whether the destruction was due to the action of substances of an enzyme-like nature present in blood. To test this possibility experiments were carried out by incubating 1 ml. of the stock solution of vitamin B₁ with boiled blood. The latter was prepared by diluting 1 ml. of oxalated blood with 2 ml. of distilled water and boiling for 20 min. The mixture was put in sterile test-tubes plugged with cotton-wool and kept at 37° for 24 hr., after which it was assayed for vitamin B₁. The results, given in Table III, show that an inactivation of the vitamin has occurred and that the extent of the destruction was approximately the same as that observed when the vitamin was incubated with fresh blood. This finding suggests that, under the conditions described in these tests, the enzymes in blood did not play any part in the destruction of vitamin B₁.

A further attempt to demonstrate the possible enzymic destruction of the vitamin was made by incubating the latter with fresh oxalated rat's blood at 37° for a shorter period (i.e. $\frac{1}{2}$ hr.). No inactivation could be detected during so short a period (see Table III).

Table III. *Comparison of the destruction of vitamin B₁ in fresh and boiled blood*

Nature of substance assayed	No. of tests	Cure of bradycardia in days (average)
1 ml. vitamin B ₁ solution + 1 ml. fresh blood	15	4.7
1 ml. vitamin B ₁ solution + 1 ml. fresh blood, after 24 hr. at 37°	10	3.4
1 ml. vitamin B ₁ solution + 1 ml. boiled blood, after 24 hr. at 37°	5	3.1
1 ml. vitamin B ₁ solution + 1 ml. fresh blood, after $\frac{1}{2}$ hr. at 37°	5	4.9

Stability of vitamin B₁ in blood at 19°. A study was made of the behaviour of vitamin B₁ in blood at a lower temperature. Sterile rat's blood was also used in these experiments and 1 ml. of the vitamin B₁ solution was added to 1 ml. of oxalated blood in test-tubes, which were plugged with sterile cotton-wool. The mixtures were allowed to stand at room temperature (about 19°) for 24 hr. after which they were assayed for vitamin B₁. The results, given in Table IV, suggest that the vitamin is stable in blood at 19° for a period of at least 24 hr.

Stability of vitamin B₁ in aqueous solution at 37°. Tests were also carried out to study the stability of the vitamin in aqueous solution (pH about 6) at 37°. Samples of the stock solution (1 ml.) were pipetted into sterile test-tubes, which were plugged with cotton-wool and kept in an incubator at 37° for 24 hr. These

Table IV. *Stability of vitamin B₁ in blood at 19°.*

1 ml. vitamin B ₁ solution + 1 ml. fresh blood, after 24 hr. at 19°	1 ml. vitamin B ₁ solution + 1 ml. fresh blood (from Table II)
Cure of bradycardia (days)	Cure of bradycardia (days)
3	4.7 (average of 15 tests)
4	
4	
5	
5	
5	
6	
Average	4.6

samples were then assayed by the bradycardia method. The results are shown in Table V. It will be seen that although the average length of cure obtained was somewhat shorter than that observed with the unheated vitamin, the difference of the two means is probably not significant. Thus no appreciable destruction of the vitamin could be detected.

Table V. *Stability of vitamin B₁ in aqueous solution at 37° for 24 hr.*

1 ml. of stock solution of vitamin B ₁	
Before heat treatment	After heat treatment
Cure of bradycardia	Cure of bradycardia
days	days
3	3
4	4
4	4
4	4
4.5	4
4.5	5
5	5
5	5
5.5	.
6	.
6	.
6	.
7	.
Mean	4.9
	4.3

DISCUSSION

It has been shown that vitamin B₁ is stable (1) in aqueous solution at 0° and (2) in blood at 19°. On the other hand it was destroyed to an appreciable extent in blood in the course of 24 hr. at 37°. Experiments also suggested that the inactivation was not due to enzymic destruction. Exposure of the vitamin in aqueous solution (at pH 6) to a temperature of 37° for 24 hr. was not accompanied by any appreciable destruction. It should be pointed out that this latter experiment is not to be regarded as a control for the experiment described in Table II, where the vitamin was incubated with blood at 37°, for the reason (among others) that there was a difference in the pH of the two media. There is thus a possibility that the slightly alkaline reaction in blood may have been responsible for the acceleration of the destruction of the vitamin. No attempt, however, has been made to control the pH (i.e. to adjust the pH to 7.3) when the vitamin was heated without blood, as it was felt that the results would be difficult to interpret on the ground that buffer salts might play a role in the destruction of the vitamin, and that a complex polyphasic system resembling that in oxalated blood cannot in any case be effectively reproduced for the purpose of control tests.

From Table II it will be seen that, as a rough approximation, the extent of the destruction of vitamin B₁ in rat's blood at 37° was about 30 %. The concentration of the vitamin in these experiments is about 1 I.U./ml. blood and it is important to emphasize that the amount of destruction will, no doubt, depend to a large extent on the concentration of the vitamin present in the medium. In this connexion it is relevant to refer to the level of vitamin B₁ normally present in blood. Preliminary experiments at present in progress in this laboratory have yielded results to show that the resting level of vitamin B₁ in blood (i.e. the concentration in blood of a normal fasting animal) of the few species hitherto examined is about 3–10 I.U./100 ml. blood. It is interesting to note that Meiklejohn [quoted by Peters, 1936] has found that in vitamin B₁ deficiency there is a marked decrease in vitamin B₁ in the blood of pigeons, from 27 γ to 5.5 γ /100 ml. (10 γ = 5 I.U.). It will be seen that the concentration of the vitamin employed in these *in vitro* studies is much higher than that normally present in blood. It is realized that the conclusions reached in the present paper concerning the destruction of vitamin B₁ in rat's blood are not necessarily true for conditions *in vivo*. If they are, it seems that the blood is one of the possible sites of destruction of the vitamin in the animal body.

SUMMARY

The stability of crystalline vitamin B₁-HCl in aqueous solution and in blood has been investigated.

In aqueous solution (*pH* about 6) the vitamin was stable at 0° for at least 3 months. No deterioration was observed when it was exposed to a temperature of 37° for 24 hr.

The vitamin was stable in blood at 19° for 24 hr. When incubated with rat's blood (*pH* about 7.3) at 37° for 24 hr., however, an appreciable destruction of the vitamin was observed. This inactivation did not appear to have been due to the action of substances of an enzyme-like nature in the blood.

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