The Thermal Destruction of Vitamin B_1

The Influence of the Concentration of Aneurin and Cocarboxylase on their own Rates of Destruction

By K. T. H. FARRER, *Research Division, Kraft Walker Cheese Co. Proprietary Ltd., Melbourne, Australia*

(*Received* 17 *June* 1948)

McIntire & Frost (1944) have drawn attention to the possibility that the rate of destruction of aneurin may be dependent upon its actual concentration in the solution studied. Using solutions of aneurin hydrochloride in distilled water, adjusted to pH 6·0 ± 0·05 with dilute NaOH or HCl, they noted that the pH fell to 5·2 during heating for 4 hr. at 100° and that as the concentration increased the rate of destruction fell.

If with McIntire & Frost's results the percentage remaining after 4 hr. heating is plotted against the logarithm of the concentration, the curve obtained is apparently a straight line (Fig. 1), and it is possible to calculate the amount of aneurin remaining after boiling an unbuffered solution at any given concentration for 4 hr. If, moreover, the percentage remaining after 4 hr. is accepted as a measure of $(a-x)$ in the unimolecular reaction equation (Farrer, 1945*a*), an approximate value of k, the velocity coefficient, may be calculated $\left(k=\frac{2\cdot303}{240}\log\left(\frac{100}{a-x}\right)\right)$. When this is done for the three concentrations of McIntire & Frost and also for a concentration of 5 μg./ml. using the percentage recovery read off from the curve in Fig. 1, the values of k are found to be significantly different for each concentration.

In the course of work on the rate of destruction of aneurin and cocarboxylase under various conditions the author has used solutions of different concentrations of these compounds as the availability of filters and other components improved and the fluorimeter which was then in use was made more sensitive. Thus, the concentrations of aneurin and cocarboxylase actually used in boiling solutions have been 5 μg./ml. (Farrer, 1941, 1945*a*), 0·8 μg./ml. (Farrer, 1947*a*, *b*), 2·5 μg./ml. (Farrer, 1945*b*) and 0·8 μg./ml. (Farrer, unpublished), respectively. In spite of these differences in concentration, which should be significant according to the figures of McIntire & Frost 1944), the results obtained have been mutually interdependent and comparable with those of Booth (1943) who used concentrations of 1 μg./ml. of aneurin and 5 μg./ml. of cocarboxylase, as confirmatory values of k determined at different times with different concentrations of aneurin and cocarboxylase have shown. It, therefore, seemed that the concentration of aneurin (or cocarboxylase) is not important when the rates of destruction of the two compounds are studied in buffered solutions.

To establish this point beyond doubt, the work recorded in this paper was undertaken,

and it was found that the actual concentration of vitamin B_1 (in either form) can be important, though not at the low concentrations used in the earlier work, and that once again the electrolyte system employed had a modifying effect.

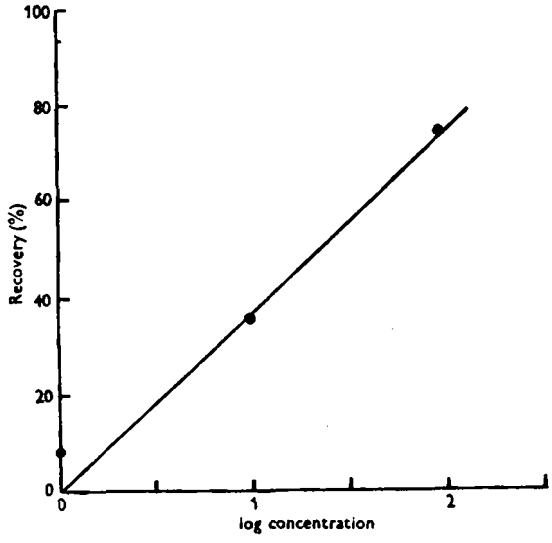

Fig. 1. Relation between recovery of aneurin after heating at 100° for 4 hr. and log concentration of aneurin; from the results of McIntire & Frost (1944).

EXPERIMENTAL

Reagents

Phosphate–phthalate solutions. A solution 0·5 M with respect to both K_2HPO_4 and potassium hydrogen phthalate was prepared and purified by the $CaCO_3$ adsorption method of Stout & Arnon (1939). After filtration, the purified solution was just acidified with 6N-HCl and boiled for 15 min. to expel carbon dioxide. On cooling, the volume was readjusted with twice-distilled water.

Disodium hydrogen phosphate. Solutions, 0·5 M, were prepared by $CaCO_3$ adsorption as described for the phosphate–phthalate solutions, but were not acidified. The carbon dioxide was expelled when the solution was mixed with citric acid to give McIlvaine (1921) buffer solutions.

Citric acid. 0·1 M solutions were prepared as described previously (Farrer, 1947*a*).

6N-*Hydrochloric acid and doubly distilled water* were prepared as described previously (Farrer, 1947*a*).

Aneurin stock solutions. Aneurin stock solutions containing 1 mg., 100 μg. and 4 μg./ml. were prepared from synthetic aneurin hydrochloride and twice-distilled water.

Cocarboxylase stock solutions. A sample of cocarboxylase supplied by Messrs Merck and Co., Rahway, N. J., U.S.A., was used. Solutions containing 1 mg. and 100 μg./ml. were prepared in twice-distilled water.

Method

The Jansen thiochrome method was used as previously described (Farrer, 1941, 1945*a*). Both the original fluorimeter and a Coleman Model 12 Photofluorometer were used to read the fluorescence.

Procedure

Solutions with a final volume of 200, 100, 50 and even 25 ml. were used as occasion demanded. Unbuffered solutions were prepared by diluting the required volume of aneurin solution with twice-distilled water and carefully adjusting to pH 6 with traces of HCl. Sufficient phosphate–phthalate solution to give final concentrations of 0·2 M and 0·02 M as required was pipetted into a volumetric flask. The requisite volume of aneurin or cocarboxylase solution to give the desired concentration of these two substances was then added and the volume made up almost to the mark. The reaction was then adjusted to pH 6 with the 6N-HCl and the solution made up to volume.

The final concentrations of the McIlvaine citric acid–Na_2HPO_4 solutions correspond to x ml. of 0·1 M-citric acid which were pipetted directly and y ml. of 0·2 M-Na_2HPO_4 which were added as an equivalent volume of 0·5 M solution (x plus y equals the final volume of the solution (cf. Britton, 1932)). Before adding the vitamin, the buffer solution was boiled to expel carbon dioxide and, on cooling, the buffer and the required volume of aneurin or cocarboxylase solution were added to the volumetric flask and made almost to volume. The pH was adjusted, if required, with 6N-HCl and the solution made up to volume.

The solutions, as specified, were boiled in flasks fitted to a reflux condenser with a ground-glass joint, and at regular time intervals samples were withdrawn and cooled rapidly. Suitable volumes of the cooled samples (usually 5 ml. but sometimes less) were diluted to give solutions containing about 1 μg./ml. of aneurin, or the cocarboxylase equivalent (or about 0·05 μg./ml. when the Coleman Photofluorometer was used). Portions containing 5 ml. of these diluted solutions were taken in triplicate for assay of aneurin. As it is necessary to hydrolyse cocarboxylase before proceeding with the assay, the measured volumes of the original samples were diluted with 10 ml. of acetate buffer at pH 4·5 (Conner & Straub, 1941) containing 50 mg. of takadiastase, and were incubated overnight at 37° before diluting to the final volume.

At the end of each experiment the pH was checked. All pH values are correct to $\pm$0·05 pH units.

RESULTS

Unbuffered solutions are most unsuitable for determinations of reaction velocity as the pH shifts appreciably during the experiment. Consequently, the results obtained in the few experiments done are recorded as percentage loss after 4 hr. boiling (see Table 1). These values generally confirm those of McIntire & Frost (1944). It is far more satisfactory to work with buffered solutions in which there are no pH changes and also to determine k, the velocity coefficient, rather than to depend on the comparison of percentage losses over a given time interval. Exactly the same procedure for determining k was used as in the earlier papers (cf. Farrer, 1945*a*). There was, however,

a difficulty with the values for the McIlvaine buffer solutions, for it will be seen from Fig. 3 that there was a steady fall in the rate of destruction as the concentration of aneurin (or cocarboxylase) in the solution was reduced by thermal destruction. This may be demonstrated from the results obtained. For example, in an experiment with McIlvaine citric acid–phosphate buffer at pH 5 and aneurin at an initial concentration of 100 μg./ml., the value of k over the 1st hr. after which 94 μg. of aneurin/ml. remained, was 0·0014. At 2 and 3 hr. there were 81 and 74 μg. of aneurin/ml., respectively, and the latter part of the log $(a-x)/t$ curve gave $k=0{\cdot}0023$. This was very close to the value ($k=0{\cdot}0022$) obtained for a similar solution in which the initial concentration of aneurin was 75 μg./ml. Accordingly, the time intervals were greatly reduced and fewer readings taken, so that not more than about 8% of the aneurin (or cocarboxylase) initially present was lost in the experiment. This reduced the errors due to changes in k to the limits of normal experimental error, and the values of k obtained, though actually approximations, must have been close to the theoretical figure for the initial concentration in each case.

Table 1. *Recovery of aneurin on boiling unbuffered solutions for 4 hr.*

Concentration of aneurin (μg./ml.)	Percentage recovery	pH	
		At 0 hr.	At 4 hr.
1	8	6·0	5·0
4	33	6·2	5·2
10	35	6·0	5·0

The results are expressed graphically in Figs. 2 and 3 in which log k is plotted against initial concentration of aneurin or cocarboxylase. It should be noted that concentrations of cocarboxylase are comparable with those of aneurin on a weight for weight basis. The effective molecular concentration is 72% of that of the aneurin.

Four experiments were done with concentrations of only 0·1 μg./ml. The results are shown in Table 2 and compared with those obtained under similar conditions at concentrations of 1·0 μg./ml. A single experiment with a concentration of aneurin of 0·01 μg./ml. in citric acid–phosphate buffer at pH 6 showed no loss of aneurin in 1·5 hr.

DISCUSSION

The claim of McIntire & Frost (1944) that, as the concentration of aneurin increases, the amount remaining after 4 hr. falls has been, in part, substantiated. However, Figs. 2 and 3 show that, to obtain a clear picture for a given set of conditions, it is necessary to study reaction rates for aneurin destruction at the various concentrations used. It is apparent that here again the exact influence of the initial concentration of the two forms of vitamin B_1 studied is a function of the buffer system used, for, with the McIlvaine buffer there is a linear relation between log k and initial concentration; but, with the phosphate–phthalate buffer, an apparent steady fall in log k with increasing concentration of aneurin or cocarboxylase gives place, at about 40 μg./ml., to a value which is unaffected by further increases in concentration.

Fig. 2 indicates that the effect of initial concentration of aneurin is not influenced by changes in concentration of buffer salts, as the curves obtained with 0·2 M and 0·02 M solutions of phosphate–phthalate buffer are parallel, the constant difference between them being the same as the difference found earlier between the two curves at pH 6.

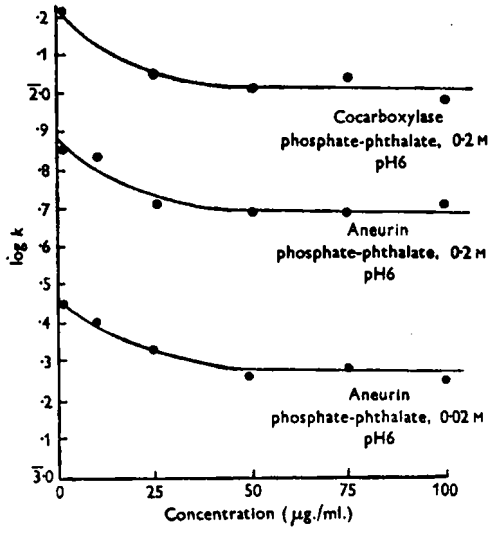

Fig. 2. Log k/concentration curves for cocarboxylase and aneurin in phosphate–phthalate buffer solutions.

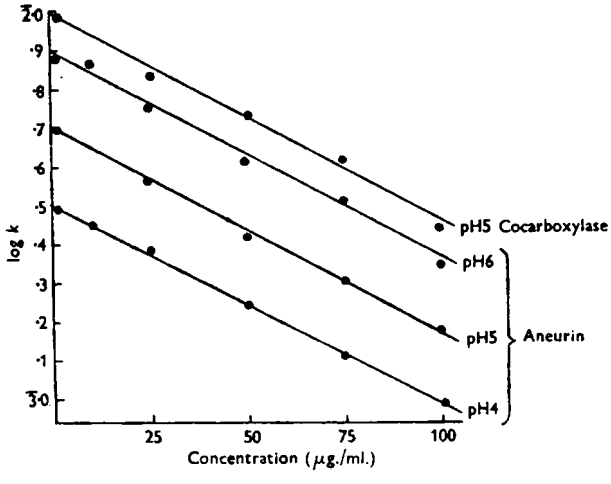

Fig. 3. Log k/concentration curves for aneurin and cocarboxylase in McIlvaine phosphate–citric acid solutions.

It will be seen from Fig. 3 that pH also is without effect on the influence of the initial concentration of aneurin. The curves at pH 4, 5 and 6 are all parallel and separated by the same amount as the values for log k obtained by Farrer (1945a) when the concentration of aneurin used was 5 μg./ml.

That cocarboxylase would parallel the behaviour of aneurin but at a faster rate was to be expected from earlier work (Farrer, 1945*b*), and work as yet unpublished on the influence of the concentration of buffer salts on the rate of destruction of cocarboxylase. This is seen to be the case both at pH 5 (Fig. 3) and pH 6 (Fig. 2), the value for $\Delta \log k$ (the difference between $\log k_C$ and $\log k_A$ (Farrer, 1945*b*)) being the same as that previously obtained for the same conditions.

Table 2. *Comparison of reaction velocities with* 1·0 *and* 0·1 *μg./ml.*

Buffer	pH	Substrate	$k_{0\cdot1}$	$k_{1\cdot0}$
Citric acid–phosphate	6	Aneurin	0·0077	0·0072
Phosphate–phthalate, 0·02 M	6	Aneurin	0·0027	0·0028
Phosphate–phthalate, 0·2M	6	Aneurin	0·0075	0·0071
Phosphate–phthalate, 0·2M	6	Cocarboxylase	0·0140	0·0160

Although in both the buffers studied there is a steady fall in $\log k$ (and hence in k) at concentrations from 1 to at least 20 μg./ml., a tenfold dilution below 1 μg./ml. is without effect on the rate of destruction. This is apparent from Table 2, in which the variations in k must be ascribed to experimental error. If this also holds in unbuffered solutions, the percentage of the original amount remaining after 4 hr. will be the same at a concentration of 0·1 as at 1·0 μg./ml., and the point at which the logarithm of the concentration is 0 in Fig. 1 will indicate a legitimate change of direction as the curve flattens out. This is supported by the confirmation of McIntire & Frost's value for percentage remaining in a 1·0 μg./ml. solution after 4 hr. boiling (see Table 1). Finally, it can be seen from Figs 2 and 3 that the differences between the values for $\log k$ at 1 and 5 μg./ml. are of the same order as the experimental error. This explains the concordance found in earlier work between results obtained with solutions of aneurin and cocarboxylase of concentrations ranging between 0·8 and 5 μg./ml.

SUMMARY

1. The influence of their own initial concentrations on the reaction velocity k for the thermal destruction of aneurin or cocarboxylase has been studied in unbuffered solutions and in solutions of McIlvaine citric acid–phosphate and of phosphate–phthalate buffers at 100°.

2. In McIlvaine citric acid–phosphate buffers there was a linear relationship between concentration of aneurin (or cocarboxylase) and $\log k$ over the range of concentration studied, 1–100 μg./ml. $\log k$ fell as the concentration of each factor increased.

3. In phosphate–phthalate solutions the $\log k$/concentration curve at first showed a gradual fall in $\log k$ with rising concentration, but above 40 μg./ml. increases in concentration of either form of vitamin B_1 (up to 100 μg./ml.) were without effect on the reaction velocity.

4. Changes in pH and concentration of the buffer salts were without effect on the relation between $\log k$ and concentration of aneurin, merely displacing the curve by an amount expected from earlier studies of these factors.

5. The rate of destruction of aneurin and cocarboxylase was unaffected by dilution from 1·0 to 0·1 μg./ml.

The author thanks the Directors of the Kraft Walker Cheese Co. Proprietary Ltd. for permission to publish this paper, and gladly acknowledges the assistance of Miss W. E. Collard in performing the many aneurin assays.

REFERENCES

Booth, R. G. (1943). *Biochem. J.* **37**, 518.
Britton, H. T. S. (1932). *Hydrogen Ions* 2nd ed., p. 219. London: Chapman and Hall.
Conner, R. T. & Straub, G. J. (1941). *Industr. Engng Chem.* (Anal. ed.), **13**, 380.
Farrer, K. T. H. (1941). *J. Proc. Aust. chem. Inst.* **8**, 113.
Farrer, K. T. H. (1945*a*). *Biochem. J.* **39**, 128.
Farrer, K. T. H. (1945*b*). *Biochem. J.* **39**, 261.
Farrer, K. T. H. (1947*a*). *Biochem. J.* **41**, 162.
Farrer, K. T. H. (1947*b*). *Biochem. J.* **41**, 167.
McIlvaine, T. C. (1921). *J. biol. Chem.* **49**, 183.
McIntire, F. C. & Frost, D. V. (1944). *J. Amer. chem. Soc.* **66**, 1317.
Stout, P. R. & Arnon, D. I. (1939). *Amer. J. Bot.* **26**, 144.