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The Thermal Destruction of Vitamin B₁4. THE INFLUENCE OF THE CONCENTRATION OF BUFFER SALTS
ON THE RATE OF DESTRUCTION OF ANEURIN AT 100°

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In the course of the work on the influence of copper (Farrer, 1947), it was noticed that aneurin was destroyed more slowly in buffer solutions considerably more dilute than those used in earlier experiments (Farrer, 1941, 1945 a, b). The influence of the concentration of buffer salts on the thermal destruction of aneurin has therefore been investigated further, with the methods already developed. It has been found that the concentration of buffer salts can, under certain conditions, greatly influence the rate of destruction of aneurin but that the effect can vary in different buffer solutions.

EXPERIMENTAL

Reagents

The work reported herein was carried out before the effect of copper upon the thermal destruction of aneurin had been completely investigated (Farrer, 1947). Consequently reagents purified by recrystallization and not CaCO₃ adsorption were used. However, this work has been allowed to stand as the solutions were copper-free and reaction velocity determinations with 0.1M-phosphate and 0.02M-phosphate-phthalate solutions purified by CaCO₃ adsorption gave results exactly comparable with those obtained with the recrystallized reagents. Moreover, the results of Farrer (1947) show that other heavy metals (Fe, Zn and Ni) have no effect on aneurin destruction in the absence of complex-forming anions, and Booth (1943) could find no effect on aneurin destruction by adding Fe, Sn, Al or Zn to phosphate-phthalate solutions.

Phosphate buffer solutions. Na₂HPO₄·12H₂O was thrice recrystallized from glass-distilled water. A 0.2M solution was prepared with glass-distilled water and diluted as required for the various concentrations. The pH was adjusted with glass-distilled 6N-HCl.

Phosphate-phthalate buffer solutions. Samples of potassium hydrogen phthalate and KH₂PO₄ were twice recrystallized from glass-distilled water and a stock solution 0.2M with respect to each salt was made up. A slight flocculent precipitate, identified as an Al salt was always obtained no matter how carefully the salts were recrystallized (cf. Clark, 1928). Repeated determinations of *k* carried out in the presence of 5 p.p.m. of Al gave the same results as were obtained in its absence (as indicated by the alizarin spot test) which confirmed Booth's result. Accordingly, the precipitate was removed by filtration. The pH was adjusted to the desired value with KOH (copper-free).

Phosphate-citrate buffer solutions. A 0.1M solution of the citric acid used by Farrer (1947) was prepared with glass-distilled water. The 0.2M-Na₂HPO₄·12H₂O solution described above was used for these solutions too.

The aneurin stock solution and the method of assay were as described by Farrer (1947).

Procedure. In the case of the phosphate-citrate buffer, 20 ml. of solution of the desired pH were prepared by pipetting the appropriate volumes of the citric acid and phosphate solutions into a 200 ml. volumetric flask; 4 ml. of aneurin solution were added, and the solution was made up to volume giving a tenfold dilution of the McIlvaine buffer used in the earlier work (Farrer, 1945a). The final pH was measured.

The preparation of the phosphate and phosphate-phthalate solutions and the subsequent procedure were the same as described by Farrer (1947).

RESULTS

The method of interpretation is the same as was used in the earlier work. For the phosphate (Fig. 1) and phosphate-phthalate (Fig. 2) solutions pH/log *k* curves are given and in Figs. 3 and 4, *k* has been plotted against the salt concentration for solutions of

constant pH. With the phosphate-citrate solutions no difference in reaction velocity at the greater dilution could be detected at pH 3, 3.5, 4, 4.5, 4.75, 5.04, 6.01 and 7.09. Hence it was not thought necessary to reproduce the pH/log k curve which has already been presented (Farrer, 1945*a*).

above pH 6 its rate of destruction appears independent of the concentration of the buffer salts within the limits studied. However, it is in the phosphate-phthalate buffer solutions that the concentration of the buffer salts is found to have the greatest effect. At all pH values between 3.9 and 7, the rate of

Fig. 1. pH/log k curves for aneurin destruction in phosphate solutions of two different concentrations (k = velocity coefficient for the thermal destruction of aneurin).

Fig. 2. pH/log k curves for the destruction of aneurin in phosphate-phthalate solutions of two different concentrations.

Fig. 3. Concentration/ k curve for the destruction of aneurin in phosphate solutions at pH 5.04 (± 0.04).

Fig. 4. Concentration/ k curve for the destruction of aneurin in phosphate-phthalate solutions at pH 5.90 (± 0.04).

It is evident from Figs. 1 and 2 that the concentration of the buffer salts affects profoundly the rate of destruction of aneurin. Thus, in direct contrast with the phosphate-citrate solutions, aneurin is destroyed progressively more slowly in the phosphate solutions below pH 6 as the pH falls, although

aneurin destruction is much less in the 0.02M solutions than in the 0.2M. In addition, the step in the pH/log k curve for the 0.2M solution has almost disappeared at the lower concentration.

The course of this variation in the rate of destruction of aneurin as the salt concentration was in-

creased was studied; pH 5 for phosphate solutions, and pH 5.9 for phosphate-phthalate solutions, were selected as convenient acidities at which to work. With both solutions, the velocity coefficient, k increases as the concentration of the solution is increased. In the phosphate solutions, the rate of aneurin destruction rises, at critical concentrations, through a series of three levels as the concentration is increased from 0.02 to 0.2M (Fig. 3). On the other hand, in the case of the phosphate-phthalate buffer, k increases continually with the concentration over the range studied (Fig. 4).

DISCUSSION

It is apparent that the behaviour of aneurin in the presence of phosphate is varied by the citrate and phthalate ions. Citrate appears to suppress the effect of concentration altogether, and phthalate has altered the relationship between concentration and reaction velocity. This serves still further to emphasize the influence of the various buffer ions on the thermal destruction of aneurin.

The concentration effect has also been found in solutions of yeast extract in which a sudden rise in reaction velocity from one level to another at an almost critical concentration was observed. The concentration/ k curve was very similar to that

found with the phosphate buffer solutions, although it consisted of only one 'step' and the values of k were of a different order from those obtained with the last-mentioned buffer at comparable concentrations.

SUMMARY

1. The effect of the concentration of the buffer salts on the rate of destruction of aneurin was studied in three different buffer solutions between the concentrations 0.2–0.02M and the pH values 3.9–7.0.

2. In phosphate buffer solutions the velocity coefficient, k , is dependent on the concentration of the buffer salts below pH 6. This effect appears to be eliminated by citrate in the phosphate-citric acid buffer solutions and magnified by phthalate in phosphate-phthalate buffer solutions.

3. In phosphate buffer solutions at pH 5, the velocity coefficient rises sharply at critical concentrations of about M/39 and M/19 through three levels when the concentration is increased from 0.02 to 0.2M. However, in phosphate-phthalate buffer solutions at pH 5.9, the relation between the velocity coefficient and concentration changes continuously over the same concentration range:

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The Effect of Age and of the Supply of Phosphorus on the Assimilation of Calcium by the Rat*

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Young growing rats retain Ca with almost 100% efficiency from diets suboptimal in this element but adequate in P (Ellis & Mitchell, 1933; Fairbanks & Mitchell, 1936; Henry & Kon, 1939). The last named authors found that under most favourable conditions the daily loss of Ca by a young rat could be as low as 0.3 mg. but that the daily excretion of P could not be brought below 1.3 mg.

* Read in part before the Biochemical Society (Henry & Kon, 1945).

In those experiments the diets contained sub-optimal but appreciable quantities of Ca and one aim of the present study was to find whether with much lower intakes this element would be held with equal tenacity, and to determine the amount of P needed to promote its most efficient utilization.

For this purpose the P content of a diet originally very low in Ca and P (diet 191) was raised by addition of Na₂HPO₄·12H₂O to a level sufficient to cover the minimum unavoidable loss of this element