THE SPECIFIC EFFECTS OF BUFFERS UPON UREASE ACTIVITY*

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Since nearly all of the previous measurements of urease activity have been carried out in the presence of phosphate buffer, it has not been generally recognized that the activity of urease is greatly influenced by the type of buffer employed; indeed the results of earlier workers are a measure of the effect of phosphate upon urease as well as a measure of the effect of urease upon urea. Krebs and Henseleit (1) state that at pH 5 phosphate buffer inhibits urease action and Folin (2) has recently preferred to employ acetate buffer in the analysis of urea by urease in blood and urine. It may be recalled that phosphate has been found to inhibit saccharase (3–5), catalase (6), peroxidase (6), and amylases (7, 8).

We have investigated the effect of acetate, citrate, and phosphate upon crystalline urease from the jack bean. With these three buffers we have obtained urea concentration curves for urease at several pH values. As is shown in Fig. 1 the activity of urease in phosphate buffer at a given pH increases until an optimum urea concentration is reached, after which the activity decreases. This effect depends upon the pH of the buffer, for when the pH is below pH 6.0 there is no inhibition of urease activity when as much as 10 per cent urea is used, while if the pH of the buffer is above pH 6.0 the amount of urea required to inhibit urease activity decreases with decreasing acidity. The

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curves also show that the urea concentration for optimum activity increases with increasing acidity. At pH 5.0 the optimum urea concentration with phosphate buffer is calculated to be about 20 per cent; at 5.6 it is 10 per cent; at 6.4 it is 5 per cent; at 6.7 it is 2.5 per cent; at 7.2 it is 1.5 per cent; and at 7.9 it is about 0.7 per cent.

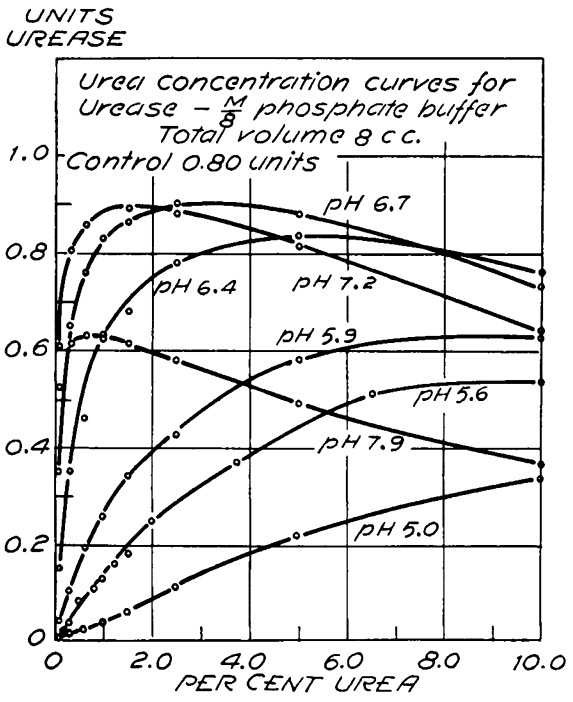

FIG. 1

The same general relationship as that described above holds for acetate and citrate buffers (Figs. 2 and 3), although with these two buffers the concentration of urea required for optimum activity is consistently lower. While with phosphate buffer optimum urease activity at optimum pH is with 2 per cent urea, in acetate and citrate buffers it is attained with 1 per cent urea.

In order to rule out the effect upon our results of buffer, or

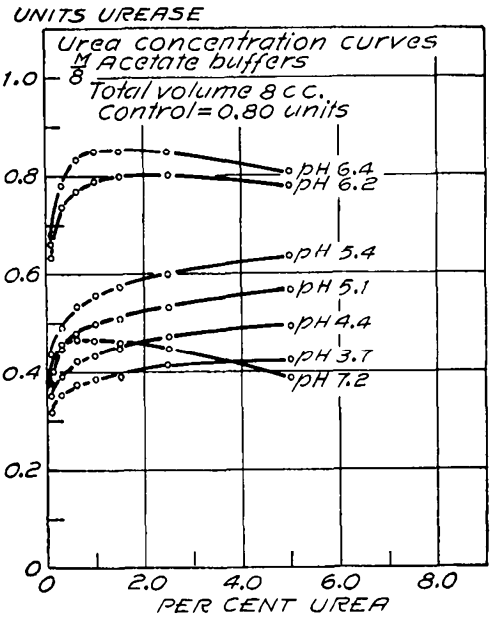
UNITS UREASE
Urea concentration curves
M/8 Acetate buffers
Total volume 8 c.c.
Control = 0.80 units
pH 6.4
pH 6.2
pH 5.4
pH 5.1
pH 4.4
pH 3.7
pH 7.2
1.0
0.8
0.6
0.4
0.2
0
0
2.0
4.0
6.0
8.0
PER CENT UREA

FIG. 2

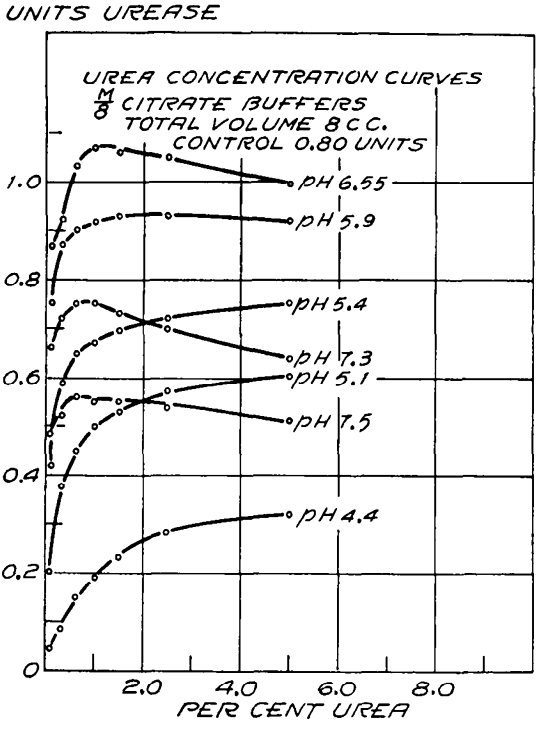
UNITS UREASE
UREA CONCENTRATION CURVES
M/8 CITRATE BUFFERS
TOTAL VOLUME 8 C.C.
CONTROL 0.80 UNITS
pH 6.55
pH 5.9
pH 5.4
pH 7.3
pH 5.1
pH 7.5
pH 4.4
1.0
0.8
0.6
0.4
0.2
0
2.0
4.0
6.0
8.0
PER CENT UREA

FIG. 3

salt concentration, we have obtained buffer dilution curves, which are shown in Fig. 4. Here two different urea concentrations were employed with the three buffers. The curves show that urease activity increases with increasing buffer dilution until a point is reached beyond which further dilution may cause the activity to decrease. With 0.1 per cent urea concentration the optimum dilution for all three buffers is M/8, but with 2.5 per cent urea it is

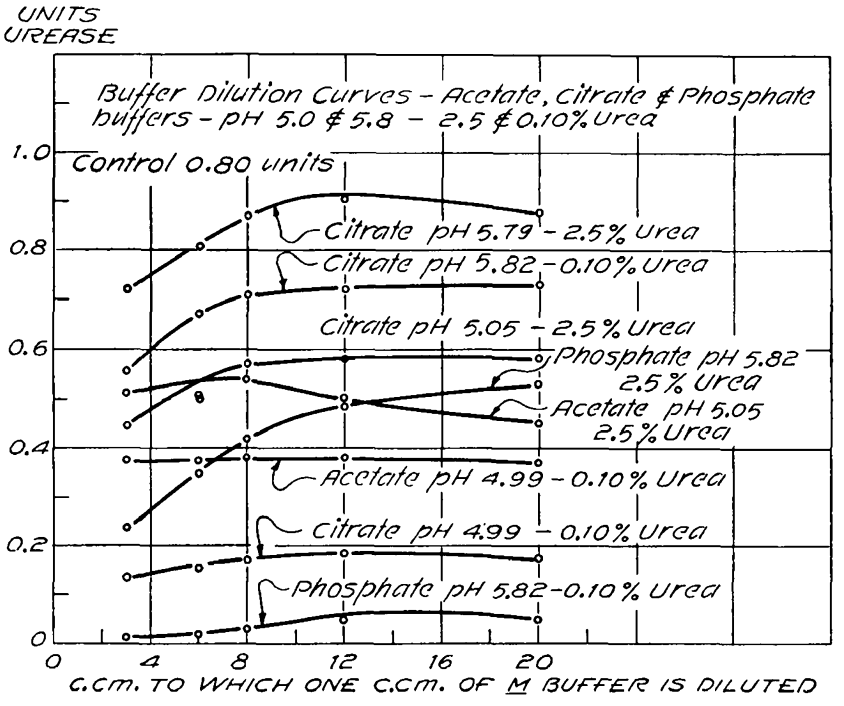

FIG. 4

M/12. Van Slyke and Zacharias (9) reported an optimum phosphate buffer dilution at M/16 at pH 7.0 with 0.91 per cent urea.

The specific effects of acetate, citrate, and phosphate buffers have been determined at various pH values, 0.1 and 2.5 per cent urea concentrations being used, as shown in Figs. 5 and 6. Here an entirely different activity-pH curve is obtained for each buffer. With phosphate urease is active from pH 5 to 9, with

citrate from pH 4 to 8.5, and with acetate from below pH 3 to 7.5. The optimum pH differs with each buffer as well as with urea concentration. With 2.5 per cent urea the optimum is at pH 6.4 for acetate, at 6.5 for citrate, and at 6.9 for phosphate; while with 0.1 per cent urea the values are shifted respectively to pH 6.7, 6.7, and 7.6. It is of interest to note that Ringer and van Trigt (10) found the pH optimum for ptyalin to be situated more towards

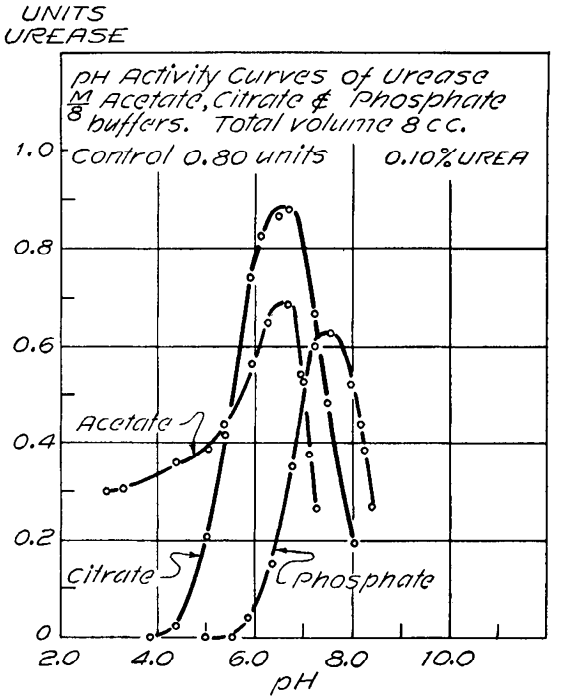

FIG. 5

the alkaline side in citrate buffer than in phosphate or acetate buffers. Hahn and his coworkers (7, 8) found the optimum for salivary amylase to be pH 5.6 in acetate buffer and 6.5 in phosphate. For pancreatic amylase the values were respectively 6.5 and 7.1. The pancreatic amylase was 3.3 times more active in phosphate than in acetate. The greatest activity of urease has been attained with citrate buffer at pH 6.5 and with a urea con-

centration of 1 per cent. In this connection it is of interest to note that the pH optima for urease at various urea concentrations have been repeatedly misstated (11–14).

It has already been noted by Van Slyke and Zacharias (9) and by Lövgren (15) that the pH optimum for urease shifts toward the alkaline side with decreasing urea concentration. Although this shift is considerable with phosphate buffer, it is slight with both acetate and citrate.

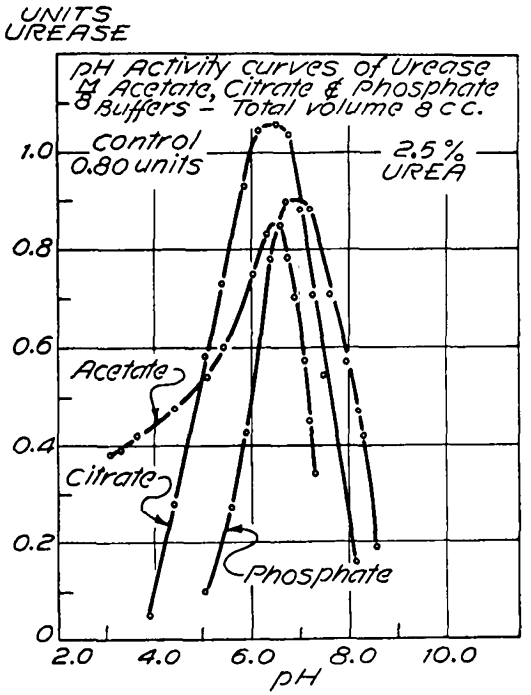

FIG. 6

The activity-pH curve for urease in acetate buffer is quite different in the extreme acid range from that of the other two buffers, for the curve with acetate shows no sharp drop with increasing acidity.

EXPERIMENTAL

For each measurement of urease activity three analyses have been run, an activity determination, a control, and a blank. In

addition the pH has been determined each time both before and after incubation with urea. Urease activity was determined by adding 1 cc. of diluted urease to 7 cc. of urea-buffer contained in a large test-tube and kept at 20° in a thermostat bath. The digestion was allowed to proceed for 1 minute and was then stopped by the addition of 3 cc. of N hydrochloric acid. The ammonia formed was determined by aeration and titration. When the determination of pH was to be made urease action was stopped by adding quinhydrone. The pH values in all cases are averages of the pH before and after urease action.

The urease used was recrystallized once from 30 per cent alcohol and stock solutions were made up containing 1200 units per cc. For daily use 1 cc. was diluted 400 times. The urea and acid potassium phosphate were especially purified by us, while other reagents were Kahlbaum's. All water used was redistilled from glass.

SUMMARY

1. The activity of urease depends upon the type of buffer present as well as upon temperature, pH, urea concentration, and salt concentration.

2. The pH optimum for urease acting upon 2.5 per cent urea is 6.4 for acetate, 6.5 for citrate, and 6.9 for phosphate. With 0.1 per cent urea the optimum is 6.7 for acetate, 6.7 for citrate, and 7.6 for phosphate.

3. The highest activity is exerted by urease in the presence of 1.0 per cent urea and M/8 citrate buffer at pH 6.5.

4. In phosphate buffer urease is active from pH 5 to 9, in citrate buffer from pH 4 to 8.5, and in acetate buffer from below pH 3 to 7.5.

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