STUDIES OF THE INFLUENCE OF COLCHICINE AND 3-INDOLEACETIC ACID UPON SOME ENZYMATIC REACTIONS

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The present investigations were undertaken with the objective of making a preliminary determination of the influence of colchicine and 3-indoleacetic acid upon the enzymatic activities of diastase and invertase. In this way it was hoped to ascertain whether the growth-stimulating property of these substances is, at least in part, directly associated with an enzymatic activation.

The diastase was prepared from commercial malt-diastase according to the method of Meyer and Anderson (1937). The invertase was obtained from two sources. The first consisted of an aqueous extract of macerated yeast cells, which were procured in ordinary dry-yeast form, and the second was a commercial extract.**

The activities of the enzymes were measured by titration with quantitative Benedict's solution***. The quantities of the hydrolysis products of starch (i. e., maltose and glucose) were assumed to be an indication of the diastase activity. Both these sugars are reducing sugars, thus making possible the Benedict's test. Likewise, the quantities of fructose and glucose obtained by the timed action of invertase on sucrose should indicate the rate of that reaction.

The test substances were introduced in concentrations varying from 1:1,000 to 1:100,000 with colchicine and from 1:3,125 to 1:100,000 with 3-indoleacetic acid. In each case doubly distilled water was used. The test substances and substrates (C. P. soluble starch and C. P. sucrose) were mixed in a common solution to which the enzymatic extract was added. To a series composed of the different concentrations of the test-substances, and the control, were added, simultaneously, equal amounts of the enzymatic extract. The time of reaction for each series is given in the tables, as well as the concentration of the substrate and the temperature of the reaction-media. Each solution was 40 cc in volume and was contained in a small beaker. The beakers were surrounded with a common water-bath in an attempt to keep the temperature of the series constant. The reactions were stopped by placing the beakers into moderately warm water and elevating the temperature of the reacting solutions to 90° C. for a period of two minutes.

Previous to conducting the quantitative experiments, it was necessary to test the concentration of the enzyme in order to regulate the rate of reaction. It was desired to stop the experiments at such a time that approximately 25 cc of the control solution would contain a sufficient amount of reducing sugar(s) to reduce 25 cc of Benedict's solution. On the basis of these preliminary tests, the enzyme extracts were diluted so that the reaction would approximate the desired point in 15 minutes.

Since the determinations were made in triplicate, each ratio number in the tables represents the average of three. Thus, actually, 15 series of tests were conducted with starch and diastase and 24 tests with sucrose and invertase.

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*** Made according to the method described by M. Bodansky and M. Fay. 1925. Laboratory manual of physiological chemistry. 3d Ed., p. 38 New York: John Wiley and Sons, Inc.

TABLE I

Diastase Tests†

Test substance		Temperature and reaction time* 26°C 16 min.	27°C 15 min.	27°C 15 min.	28°C 14 min.	30°C 15 min.	Average
Control	V**	100.0	100.0	100.0	100.0	100.0	100.0
	pH	6.3	6.4	6.4	6.4	6.3	
3-Indole-acetic acid							
1:50,000	V	99.2	100.4	103.9	100.0	103.9	101.5±0.52
	pH	5.7	5.6	5.5	5.6	5.6	
1:25,000	V	100.6	99.8	110.8	105.8	108.4	105.1±1
	pH	5.3	5.2	5.2	5.2	5.2	
1:12,500	V	104.5	103.3	111.4	108.4	112.9	108.1±1
	pH	4.9	4.8	4.8	4.9	4.9	
1:6,250	V	110.9	108.3	118.8	117.3	116.9	114.4±1
	pH	4.5	4.4	4.4	4.4	4.4	
1:3,125	V	119.8	120.1	123.1	123.2	120.4	121.3±0.4
	pH	4.3	4.2	4.2	4.2	4.2	
Colchicine							
1:100,000	V	94.0	95.4	75.1	74.2	76.6	84.0±2.5
	pH	6.4	6.4	6.4	6.5	6.4	
1:10,000	V	89.9	91.5	73.8	69.3	69.9	78.9±2.5
	pH	6.5	6.5	6.5	6.5	6.5	
1:1,000	V	77.2	78.7	67.6	62.3	65.6	70.3±1.7
	pH	6.7	6.7	6.7	6.7	6.7	

† Substrate, 2 percent starch solution.

* The vertical columns represent series which were run concurrently, with equal amounts of enzyme; thus comparisons should be made within these columns.

** V, the volume of control solution that reduced 25 cc of Benedict's solution, is considered to be 100 in each series and the volumes of the other solutions are expressed accordingly. It is important to note that each volume in the table is an average of triplicate tests, making a total of 15 tests considered in evaluating the standard error. All values of V significantly less than 100 indicate that the rate was greater than the rate in the control; likewise, all those above 100 indicate a decrease in reaction rate.

The results of the diastase tests (Table I) show that 3-indoleacetic acid accelerates the action of this mixture of enzymes upon starch. A 1:50,000 concentration has little noticeable effect. However, as the concentration of 3-indoleacetic acid is increased, there is a decided tendency toward inhibition of the reaction. Presumably this is a response caused by the pH change and not by the accumulation of indoleacetate radicles. This is consistent with the information given by Waldschmidt-Leitz and Walton (1929) that the optimum pH range for maltase action is from 6.1 to 6.8 and that for malt amylase action is from 4.6 to 5.2 (presumably these determinations were made at room temperature). The pHs of the solution at concentrations of 1:6,250 and 1:3,125 were 4.4 and 4.2, respectively, (except in the runs at 26°, in which they were 4.5 and 4.3), and, since these are below the optima, the inhibition can logically be attributed to the unfavorable pH.

The results indicate that colchicine is a favorable constituent of the reaction-medium, causing a definite increase in the rate of reaction. The reaction-rate increases with the increase in the concentration of the colchicine. The maximum diastase activity in these tests occurred in the 1:1,000 colchicine series at pH 6.7. Although this pH is within the optimum range for the action of maltase, it is farther from the optimum (4.6 to 5.2) for the activity of amylase than the control solutions were. Thus, apparently, more is involved than the response to a pH-change. In view of the fact that the rate is correspondingly greater in each case of increase of colchicine

TABLE II
Invertase Tests†

Test substance		Temperature and reaction time*								Average
		27°C 15 min.	26°C 15 min.	26°C 15 min.	27°C 21 min.	35°C 15 min.	33°C 14 min. ††	27°C 20 min. ††	30°C 18 min. ††	
Control	V**	100.0	100.00	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	pH	6.0	6.1	6.0	6.0	6.1	6.4	6.4	6.4	
3-Indole-acetic acid										
1:100,000	V	.....	86.4	91.4	92.1	90.7	70.3	58.5	61.5	78.7±3
	pH	.....	5.4	5.4	5.5	5.6	6.1	5.8	5.7	
1:50,000	V	95.5	86.2	87.7	92.1	89.0	59.1	48.4	47.9	75.8±3.9
	pH	5.3	5.2	5.2	5.2	5.3	5.4	5.4	5.3	
1:25,000	V	95.2	86.2	84.8	93.7	90.4	54.3	42.7	56.4	75.5±4
	pH	5.1	5.0	5.0	5.1	5.1	4.9	4.9	4.8	
1:12,500	V	77.9	78.0	83.3	91.3	90.1	70.6	46.3	56.4	74.2±3
	pH	4.8	4.8	4.8	4.9	4.8	4.6	4.7	4.6	
1:6,250	V	95.1	87.2	89.6	94.5	93.3	64.2	53.7	60.3	79.7±3.3
	pH	4.6	4.6	4.6	4.6	4.5	4.3	4.3	4.3	
1:3,125	V	101.6	92.2	93.7	103.1	95.7	76.6	57.8	58.9	84.9±3.5
	pH	4.3	4.3	4.2	4.3	4.2	4.0	4.0	3.9	
Colchicine										
1:50,000	V	100.1	100.9	100.0	99.5	100.9	99.0	97.8	100.5	99.8±.2
	pH	6.1	6.1	6.1	6.1	6.1	6.4	6.4	6.4	
1:10,000	V	101.1	104.0	102.2	103.9	105.6	106.7	104.9	102.8	103.9±.4
	pH	6.2	6.2	6.2	6.2	6.2	6.5	6.5	6.5	
1:1,000	V	104.9	110.7	106.7	110.5	113.7	.....	.....	.....	109.3±.8
	pH	6.4	6.4	6.3	6.4	6.4	.....	.....	.....	

† Substrate, 5 per cent sucrose solution in all trials except those represented by the first column of results (27° C, 15 min.), in which 2 per cent sucrose solution was used.

* See corresponding footnote to Table I

** See corresponding footnote to Table I, but note that 24 results instead of 15 are averaged.

†† Commercial invertase extract used.

it is suggested that the acceleration may be due to colloidal action of the drug. This necessitates the assumption that the large, alkaloidal molecules of colchicine affect the process of adsorption. Also, it may be assumed that the adsorption of the substrate onto the surface of the colloidal molecules would increase the reaction-surface of the system and thereby bring about the stimulation. However, the stimulation may be due to the adsorption of water by the colchicine, with consequent concentration of the reacting substances. The "adsorbability" of the starch particles may be influenced. The effect, however, is not produced by the colchicine alone, for in those cases where the enzyme was inactivated by heat no reduction of Benedict's solution was obtained.

It is of interest to note that the results were verified by the iodine test method, with the colchicine series losing the dark blue-black color more quickly, the 3-indoleacetic acid series less quickly, than the control.

With the invertase experiments the results (Table II) were reversed, indicating that the effects must be somewhat different than in the case of diastase. Here considerable acceleration of the enzymatic hydrolysis was achieved in the 3-indoleacetic acid series and a slight inhibition in the colchicine series. Since the optimum pH for invertase activity is generally conceded to be near 4.6, it seems quite probable that the acceleration by 3-indoleacetic acid is primarily a response to the adjustment of the pH, which approaches the optimum point. This is substantiated by the evidence that the strongest concentration of this substance (1:3,125) was less efficient as a stimulus than any of the smaller concentrations and at the same time had a pH which was below the optimum point. The pH of the solutions at this strongest concentration varied from 3.9 to 4.3. The possibility exists that there is some catalytic action on the part of 3-indoleacetic acid, since 1:100,000 appears to be almost as stimulative as any other concentration.

The inhibiting effect of colchicine is small and seems to be essentially one of less favorable pH than in the control.

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SUMMARY

1. In these experiments, colchicine was found to cause an acceleration of the hydrolysis of starch by malt diastase. The method of stimulation is unknown.

2. Colchicine, in dilute solutions, appeared to have little effect upon the rate of inversion of sucrose by invertase. The inhibitory effect of strong concentrations seems to be due to the change of pH.

3. In these experiments, 3-indoleacetic acid was an unfavorable constituent of the reaction media for starch hydrolysis by malt diastase. Presumably this can be attributed to the accompanying change of pH.

4. 3-Indoleacetic acid was a favorable constituent in every concentration used in the inversion of sucrose. However, concentrations above and below 1:12,500 were less stimulative than that concentration. This indicates that the stimulation is primarily due to the pH-change, although the small difference between the 1:12,500 and the 1:100,000 series may indicate that, in dilute solutions at least, the stimulation is more than a pH-response.

LITERATURE CITED

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