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Changes in Leaf-Sugar Content and Enzyme Activity of Immature Sugarcane Following Foliar Application of Indole-3-Acetic Acid, 2,4-Dichlorophenoxyacetic Acid, and Maleic Hydrazide

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INTRODUCTION

Although recent investigations have shown that the sugar content of plants can be increased by 2,4-dichlorophenoxyacetic acid (*4,26*)[2] and by maleic hydrazide (*8,16,18,21*), workers in Puerto Rico have not been able to obtain similar responses with sugarcane (*13,14,15*).[3] Such conflicting results stem partly from the fact that the mode of action of hormonelike compounds in the area of sugar metabolism remains almost totally obscure.

Proposals advanced to account for the action of growth-regulators include the two-point attachment theory of Muir and Hansch (*17*), and the theory of Van Overbeek (*20*), which requires that the active substance possess an aromatic ring, or "anchor", and an acid group positioned outside the ring to serve in an oscillating hydrogen-bond network. Whatever the physical configuration of the regulator molecule, it is highly probable that the growth-regulating effects are brought about through reactions with endogenous enzyme systems. The potential interrelationships of auxins and enzymes have been discussed at length by Crafts (*7*), and Wort (*27*) lists 18 enzyme systems the activities of which are known to be affected by growth-regulating materials.

Proceeding with the hypothesis that plant growth-regulators may greatly affect the action of sugarcane enzymes, a new series of studies has been initiated at this Station to clarify the roles of growth regulators in sugar metabolism. The initial work reported herein deals with the effects of IAA, 2,4-D, and MH on sugar-metabolizing enzymes from leaves of immature sugarcane.

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[2] Italic numbers in parentheses refer to Literature Cited, pp. 33–4.

[3] For convenience the growth regulators discussed throughout this report are abbreviated as follows: 2,4-dichlorophenoxyacetic acid (2,4-D); maleic hydrazide (MH); indole-3-acetic acid (IAA).

MATERIALS AND METHODS

One-eye cuttings of the variety P.R.980 were planted in 13-inch clay pots containing a Vega Alta clay loam soil which had been steam-sterilized for 6 hours. The cuttings were treated for 10 minutes with 0.03-percent PMA (phenyl mercury acetate) solution. All plants were watered once daily with approximately 2 liters of tapwater per container, and each container received 1 liter of nutrient supplement at intervals of 3 days.[4] The plants were maintained in a greenhouse for the duration of the study, receiving a weekly application of Malathion-50 for the control of insects.

At 10 weeks of age 36 of the most uniform containers were selected for treatment. These were divided into four sets of nine containers each, and each group was temporarily moved outside the greenhouse where it received its respective spray application. One group received a 0.175-percent solution of IAA, the second group a 0.20-percent solution of 2,4-D, and the third group a 0.15-percent solution of MH. IAA and 2,4-D were provided as the pure acids and MH as the pure hydrazide of maleic acid.[5] The solutions were made up with distilled water by the method of Ballantyne (*3*), including 500 p.p.m. of glucose, 250 p.p.m. of boric acid, and Tween 20 as a wetting agent at the rate of 0.1 ml. per 100 ml. of solution. The control group received glucose, boric acid, and Tween 20 in distilled water. Each solution was applied with a Hudson 7.6-liter hand-sprayer at approximately 40 pounds pressure with the nozzle adjusted for a broad, fine-mist pattern. All above-soil areas of the plants were sprayed with the respective materials until thoroughly wet, and run-off had begun. For each treatment this required almost exactly 1.2 liters of solution. The plants were arranged in a completely randomized design with three replicates for each of the four treatments.

Leaf samples were taken for sugar and enzyme assay at 1, 3, 9, and 27 days following spray application. The original experimental plan called for leaf samples to be taken within an hour after treatment, but this harvest was omitted because of unforeseen difficulty in obtaining Dry Ice (solid carbon dioxide) from the local supplier.

Each sample consisted of leaves −1 to +1 from two representative plants.[6] At 10 weeks of age most plants were bearing no more than one or

[4] The nutrition supplement contained the following nutrients expressed as milliequivalents per liter: Nitrate, 10; phosphate, 6; potassium, 5; calcium, 3; magnesium, 2; and sulfate, 2. Microelements, expressed as parts per million, were supplied as follows: Iron, 1.0; boron, 0.05; copper, 0.02; manganese, 0.50; zinc, 0.05; and molybdenum, 0.01.

[5] All biochemicals employed during the study were supplied by the Nutritional Biochemicals Corp., Cleveland 28, Ohio.

[6] The leaf nomenclature employed here is that of Kuijper (*11*), in which the highest leaf bearing a visible dewlap is designated +1.

two visible dewlaps and no attempt was made to sample sheath material. After trimming the leaves with scissors, the entire blades were inserted in stoppered 38 × 200-mm. pyrex tubes, and the samples were immediately frozen in a mixture of Dry Ice and acetone. The frozen tissues were stored in a freezer at −20° C. and, over a period of several weeks, were withdrawn to a cold chamber where they were dried under vacuum, at 0–2° C., with the aid of a Virtis Roto-Freeze drying apparatus. When thoroughly dry the samples were ground at 0° C. with a Wiley Mill to pass a 60-mesh screen (0.42 mm.). The powdered leaf material was then stored at 0° C. in sample jars fitted tightly with screw tops and sealed with plastic tape until extractions could be made at our convenience.

Employing distilled-water extracts of the leaf tissue, total ketoses were determined by the resorcinol technique of Roe (*22*), and total reducing sugars were measured by the dinitrosalicylic acid method of Sumner (*24*). The hot-alkali technique of Cardini *et al.* (*6*) was used to distinguish between fructose and sucrose, and it enabled us to measure the nonsugar-reducing power of the extracts. Enzyme protein was extracted with distilled water and precipitated with ammonium sulfate as described previously (*2*). Protein was measured colorimetrically by the method of Sutherland *et al.* (*23*).

Enzyme assays were conducted for acid phosphatase, ATP-ase, invertase, amylase, hexokinase, phosphohexose isomerase, aldolase, triose phosphate dehydrogenase, phosphoglyceryl kinase, condensing enzyme, isocitric dehydrogenase, peroxidase, and transaminase. Acid phosphatase *per se* was measured with β-glycerophosphate serving as a representative substrate, but the action of specific phosphatases was also determined against glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, 3-phosphoglyceric acid, and uridine diphosphate glucose (UDPG). With the exception of UDPG-ase, details of the sugar and enzyme assays were reported previously (*2*).

UDPG-ase was included in this study on the basis of earlier work which indicated that one or more acid phosphatases were playing important roles in the alteration of the sucrose content of cane leaves. The reaction mixture for UDPG-ase was composed of 0.5 ml. of acetate buffer (pH 4.65), 0.5 ml. of 2.7×10^{-3} M UDPG solution, and 0.25 ml. of a leaf-protein solution prepared as previously reported (*2*). Control tubes received distilled water in place of enzyme. The reaction proceeded 10 minutes at 30° C. and was terminated with 1 ml. of 10-percent trichloroacetic acid. Inorganic phosphate released by enzymatic hydrolysis was determined colorimetrically by the phosphomolybdic acid technique employed for each of the other phosphatase assays.

All data were subjected to statistical analysis of variance and mean values were compared by the Student-Newman-Keuls Q test.

RESULTS

LEAF SUGARS

Maximum sucrose, fructose, and total reducing-sugar contents were recorded 9 days after spray application (table 1). Sucrose was particularly

TABLE 1.—*Mean values for leaf sugars of immature sugarcane following spray application of indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and maleic hydrazide (MH)*[1]

Sugar	Days following treatment	Result under treatment indicated				Mean
		Control	IAA	2,4-D	MH	
Total ketose	1	16.61	19.14	14.39	19.46	17.40
	3	15.58	22.18	17.34	14.68	17.44
	9	26.10	31.70	39.80	29.13	31.69
	27	16.60	17.67	14.00	19.73	17.00
Mean		18.72	22.67	21.38	20.75	20.88
Sucrose	1	14.03	14.47	10.68	14.28	13.37
	3	11.33	11.05	15.01	10.37	11.94
	9	15.93	25.64	29.13	17.60	22.07
	27	15.80	17.23	11.60	17.90	15.63
Mean		14.27	17.09	16.61	15.04	15.75
Fructose	1	2.57	4.67	3.70	5.18	4.03
	3	4.25	6.07	2.33	4.31	4.24
	9	10.16	9.71	10.00	11.63	10.37
	27	.77	.43	2.40	1.83	1.36
Mean		4.44	6.22	4.61	5.74	5.20
Total reducing	1	10.73	8.25	9.98	11.35	10.08
	3	5.53	10.31	6.77	6.80	7.35
	9	10.95	10.23	11.43	11.17	10.94
	27	4.87	5.93	5.53	9.70	6.51
Mean		8.02	8.68	8.43	9.76	8.72
Glucose	1	8.16	3.58	6.27	6.17	6.04
	3	1.28	.59	4.45	2.53	2.21
	9	1.70	4.17	1.83	1.17	2.22
	27	4.10	5.50	3.13	7.83	5.14
Mean		3.81	3.46	3.92	4.43	3.91

[1] Sugars are expressed as milligrams per gram dry weight. Each figure represents the computed mean of 3 replicates.

affected by IAA and 2,4-D, increasing by 1.6 and 1.8 times, respectively, over control values at 9 days (fig. 1). By 27 days the enhancing effect of IAA had practically disappeared and sucrose content was lower among 2,4-D-treated plants than controls. Maleic hydrazide had caused only a slight sucrose increase at 9 days, but this response was still apparent at the end of the study. Glucose content was understandably highest 1 day after treatment, since *d*-glucose was a component of the spray material. It is evident, however, that glucose was being metabolized far more rapidly among IAA-

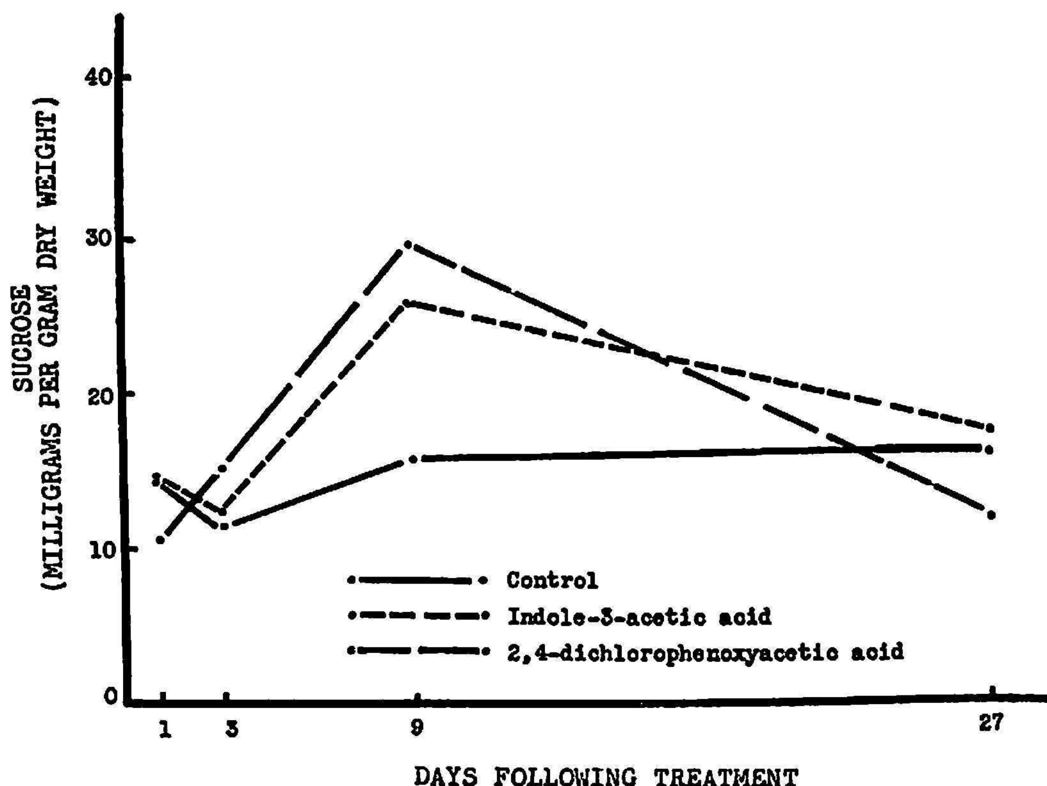


FIG. 1.—Sucrose levels in leaves of immature sugarcane following spray application of indole-3-acetic acid and 2,4-dichlorophenoxyacetic acid.

treated plants at this period than among any other treatment group, and after 3 days glucose was virtually undetectable in leaf extracts from the IAA series. The reappearance of glucose among the IAA group at 9 days reaffirms that an initial emphasis toward hexose metabolism had slackened, and that conditions were more favorable for sucrose synthesis.

A review of table 1 indicates that the increased sucrose content among the IAA and 2,4-D groups at 9 days was not due to a simple reassembly of available reducing sugars, as one might have expected. Total reducing sugars failed to decline at 9 days when sucrose increased, and they decreased by 40 percent between 9 and 27 days when the sucrose decline might logically have provided an increased supply of reducing sugars.

Further examination of table 1 reveals that the loss of reducing sugar at 27 days is wholly accounted for by the disappearance of fructose. It appears that the effects of IAA and 2,4-D upon sucrose were not manifested through control of a sucrose-reducing sugar equilibrium, but rather through control of the equilibrium between fructose and glucose. It is evident that at 27 days much of the fructose which would have appeared in sucrose earlier, as well as most of the free fructose, was now being rapidly metabolized. It follows that sucrose must not have increased at 9 days as a main effect of IAA and 2,4-D, but rather was permitted to form as a secondary reaction at a time when the plant's requirement for hexose metabolism was temporarily slackened.

ENZYME ASSAYS

The application of IAA had a general stimulating effect upon the enzymes (table 2), while MH generally inhibited enzyme action or had little effect at all. Mean values for all harvests indicate that of the 20 systems measured, IAA stimulated 15 enzymes and retarded only 1, the condensing enzyme. The 2,4-D treatment stimulated six and retarded two. Maleic hydrazide retarded eight enzymes and stimulated only three. Of the 15 systems increased by IAA, 11 exhibited the effect within 1 day after treatment, while increases by the remaining four enzymes were not recorded until 9 days or more after treatment. Of the eight systems retarded by MH, seven exhibited the effect within 1 day of treatment. One enzyme, phosphoglyceryl kinase, showed the effects after 3 days had passed.

The Acid Phosphatases

These enzymes were generally stimulated by IAA. Each of the phosphorylated substrates tested was increasingly hydrolyzed by the respective phosphatase within 1 day of IAA treatment (table 3). On the other hand, 2,4-D did not appreciably stimulate any phosphatase at the 1- or 3-day harvests, and, for 3-phosphoglyceric acid, the stimulation was not recorded before the 27-day harvest (fig. 2). The enzymes hydrolyzing β-glycerophosphate and glucose-6-phosphate showed varying degrees of decline at 9 days (table 4, items 1 and 5), which corresponded with the period of maximum sucrose, while the fructose phosphatases and 3-phosphoglyceric acid phosphatase failed to exhibit this suppression (table 3).

Glucose Oxidase

Glucose oxidase was most active at the 1-day harvest when substrate concentrations were understandably highest. The capacity of this enzyme to regulate the supply of free glucose must bear heavily on the equilibrium between sucrose synthesis and hexose metabolism. An excessive supply of

free glucose would logically favor glycolysis and the employment of ATP reserves in the metabolism of glucose. An active glucose oxidase should eventually offset such a trend, and in this respect we note that glucose-oxidase activity increased markedly at 9 days among the IAA and 2,4-D treatments which were most readily forming sucrose at this time. A comparison of grand mean values for glucose (table 1) and for glucose oxidase (table 3, item 20) indicates that the highest enzyme activity corresponds to the lowest glucose content.

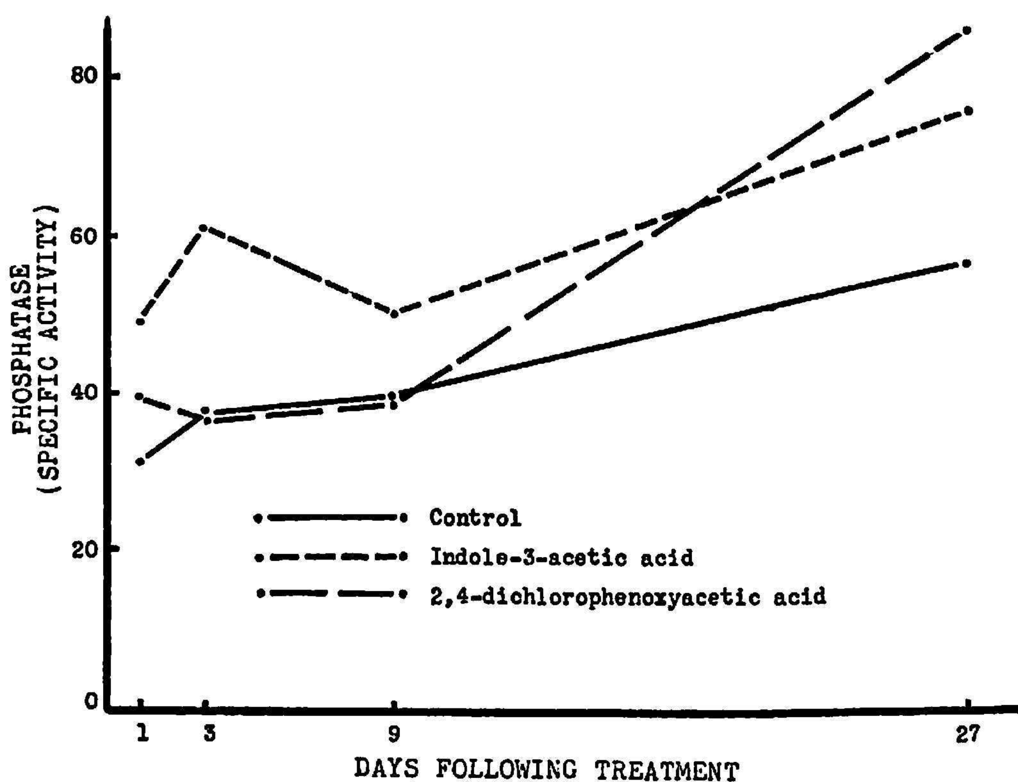


FIG. 2.—Effects of indole-3-acetic acid and 2,4-dichlorophenoxyacetic acid on the enzyme 3-phosphoglyceric acid phosphatase from leaves of immature sugarcane.

Hexokinase

The enzyme catalyzing the transfer of phosphate from ATP to glucose, hexokinase, was stimulated by each of the three compounds tested. This effect was most pronounced at the 1-day period.

Amylase, Isocitric Dehydrogenase, Phosphoglyceryl Kinase

The action of amylase and isocitric dehydrogenase was promoted by IAA and retarded both by 2,4-D and maleic hydrazide. The effect of IAA on amylase was most unusual in that the enzyme lost over half its activity between the 1- and 3-day periods, while still retaining a stimulated action by comparison with controls (fig. 3). Isocitric dehydrogenase was stimulated

by IAA primarily among the 27-day samples, whereas MH retarded the enzyme at the 1- and 3-day periods. Figure 4 illustrates the fact that 2,4-D and MH were suppressing phosphoglyceryl kinase at the 3- and 9-day harvests, and that this enzyme was later stimulated by 2,4-D.

TABLE 2.—*Comparative effects of indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and maleic hydrazide (MH) on leaf enzymes of immature sugarcane*[1]

Enzyme	Effect of treatment with—		
	IAA	2,4-D	MH
β-Glycerophosphatase	+	0	0
UDPG-phosphatase	+	0	–
ATP-ase	+	0	0
Glucose-1-phosphatase	+	0	–
Glucose-6-phosphatase	+	0	–
Fructose-6-phosphatase	+	+	0
Fructose-1,6-diphosphatase	+	0	0
3-PGA phosphatase	+	+	0
Hexokinase	+	+	+
Invertase	0	0	0
Amylase	+	–	–
Aldolase	0	0	–
Peroxidase	+	+	–
Isocitric acid dehydrogenase	+	–	–
Triose phosphate dehydrogenase	0	0	0
Transaminase	+	+	0
Phosphohexose isomerase	+	0	+
Phosphoglyceryl kinase	0	0	–
Condensing enzyme	–	0	+
Glucose oxidase	+	+	0

[1] The symbols +, 0, and – refer to enzyme stimulation, no effect, and suppression, respectively. The criteria for enzyme stimulation or suppression was abritrarily established at 20-percent deviation of mean values from the mean control term for each enzyme.

Peroxidase

Peroxidase activity was tripled by IAA within 1 day of application, and the enhancing effect of the auxin was retained through the remainder of the study (fig. 5). Maleic hydrazide inhibited the enzyme at the first two sampling periods, but this effect was no longer apparent at 9 and 27 days. Peroxidase has been of particular interest to us since the enzyme revealed an almost perfect inverse relationship with leaf sucrose in a previous study (*2*). A somewhat similar relationship was again observed during these ex-

TABLE 3.—*Specific activity values for leaf enzymes of immature sugarcane following spray application of indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and maleic hydrazide (MH)*[1]

Item	Days following treatment	Effect of treatment indicated				Mean
		Control	IAA	2,4-D	MH	
1, β-Glycerophosphatase	1	18.8	26.8	21.8	18.1	21.4
	3	21.7	34.7	20.6	16.0	23.3
	9	19.2	13.4	11.3	28.6	18.1
	27	31.5	43.5	48.2	33.4	39.1
Mean		22.8	29.6	25.5	24.0	25.5
2, ATP-ase	1	16.5	17.0	16.1	12.1	15.4
	3	24.3	42.3	23.6	17.5	26.9
	9	26.3	26.9	20.4	38.0	27.9
	27	40.7	56.0	64.5	41.3	50.6
Mean		26.9	35.6	31.1	27.2	30.2
3, UDPG phosphatase	1	4.7	6.9	4.8	3.9	5.1
	3	3.9	4.8	3.7	2.8	3.8
	9	4.4	4.0	3.6	3.9	4.0
	27	5.9	6.3	5.8	4.0	5.5
Mean		4.7	5.5	4.5	3.6	4.6
4, Glucose-1-phosphatase	1	8.6	11.2	7.4	5.9	8.3
	3	5.0	7.2	6.0	3.7	5.4
	9	5.9	6.6	4.5	4.3	5.3
	27	9.2	12.1	10.4	8.6	8.1
Mean		7.2	8.8	7.1	5.6	7.2
5, Glucose-6-phosphatase	1	5.6	8.2	5.9	4.6	6.1
	3	15.8	26.6	18.2	13.9	18.6
	9	13.6	5.2	4.2	5.6	7.1
	27	27.2	34.2	30.9	20.7	28.2
Mean		15.4	58.1	14.8	11.2	14.9
6, Fructose-6-phosphatase	1	10.4	20.6	13.4	10.0	13.6
	3	30.2	44.2	33.2	28.7	34.1
	9	29.7	47.2	48.4	44.1	42.4
	27	51.3	52.6	50.2	21.8	44.0
Mean		30.0	41.2	36.3	26.2	33.4

[1] Each figure represents the computed mean of 3 replicates.

TABLE 3.—*Continued*

Item	Days following treatment	Effect of treatment indicated				Mean
		Control	IAA	2,4-D	MH	
7, Fructose-1,6-diphosphatase	1	41.2	44.2	19.9	19.9	31.0
	3	19.2	41.1	22.0	14.4	24.2
	9	73.2	74.9	93.0	79.5	80.2
	27	85.3	113.1	98.7	68.3	91.3
Mean		54.7	68.3	58.4	45.5	56.7
8, 3-PGA phosphatase	1	31.9	49.4	39.8	23.7	36.3
	3	37.2	61.6	36.3	35.0	42.5
	9	39.6	50.2	38.4	53.3	45.4
	27	56.1	75.8	85.7	53.8	67.9
Mean		41.2	59.3	50.0	41.5	48.0
9, Hexokinase	1	16.8	47.4	39.8	34.2	34.6
	3	11.9	15.6	16.7	17.3	15.4
	9	40.9	46.2	52.2	46.3	46.4
	27	10.9	10.0	10.8	19.0	12.7
Mean		20.1	29.8	29.9	29.2	27.2
10, Invertase	1	5.0	5.8	5.3	3.5	4.9
	3	4.6	4.3	3.7	4.2	4.2
	9	4.3	3.3	4.3	3.9	3.9
	27	4.3	3.9	3.3	4.8	4.1
Mean		4.6	4.3	4.2	4.1	4.3
11, Amylase	1	226.5	358.2	213.0	168.8	241.6
	3	117.2	152.1	108.3	126.4	126.0
	9	161.8	194.6	106.5	131.2	148.5
	27	180.2	246.3	183.7	150.4	190.1
Mean		171.4	237.8	152.9	144.2	176.6
12, Aldolase	1	39.4	31.1	38.8	35.8	36.3
	3	19.8	18.2	21.3	24.9	21.0
	9	31.6	34.5	28.9	22.0	29.2
	27	21.6	26.6	13.7	7.2	17.3
Mean		28.1	27.6	25.7	22.5	26.0
13, Peroxidase	1	1.2	3.6	1.1	0.8	1.7
	3	3.9	7.6	4.4	.9	4.2
	9	1.3	2.4	1.7	1.2	1.6
	27	2.4	5.4	4.1	2.6	3.6
Mean		2.2	4.7	2.8	1.4	2.8

TABLE 3.—*Concluded*

Item	Days following treatment	Effect of treatment indicated				Mean
		Control	IAA	2,4-D	MH	
14, Isocitric acid dehydrogenase	1	23.2	33.9	17.3	15.9	22.5
	3	19.9	16.0	15.5	14.5	16.5
	9	16.6	16.9	12.8	14.2	15.1
	27	9.9	21.4	10.0	9.9	12.8
Mean		17.4	22.0	13.9	13.6	16.7
15, Triose phosphate dehydrogenase	1	4.6	3.2	4.8	5.4	4.5
	3	.9	1.8	2.2	1.5	1.6
	9	3.7	1.9	1.6	3.0	2.6
	27	1.1	2.2	1.7	2.2	1.8
Mean		2.6	2.3	2.6	3.0	2.6
16, Transaminase	1	29.6	49.4	36.4	29.4	36.2
	3	55.1	52.7	55.2	57.0	55.0
	9	43.1	63.9	58.7	53.3	54.5
	27	79.9	110.9	101.5	81.8	93.5
Mean		51.9	69.2	63.0	55.4	59.9
17, Phosphohexose isomerase	1	34.6	32.7	23.4	5.6	24.1
	3	20.9	18.3	39.4	27.1	26.4
	9	25.7	47.0	31.7	21.7	31.5
	27	41.6	53.5	12.4	98.5	51.5
Mean		30.7	37.9	26.7	38.2	33.4
18, Phosphoglyceryl kinase	1	10.4	10.8	9.7	10.5	10.4
	3	4.4	5.4	1.1	1.8	3.2
	9	9.7	13.1	3.9	3.1	7.4
	27	2.9	2.1	7.8	.6	3.3
Mean		6.9	7.9	5.6	4.0	6.1
19, Condensing enzyme	1	96.1	16.3	126.5	166.6	101.3
	3	34.4	23.3	17.4	29.7	26.1
	9	39.2	58.5	31.2	100.2	57.3
	27	32.2	33.6	25.2	40.9	33.0
Mean		50.2	33.0	50.1	84.2	54.4
20, Glucose oxidase	1	44.4	90.5	114.0	48.8	74.4
	3	37.3	43.1	29.4	23.2	33.3
	9	39.7	70.7	64.3	51.3	56.5
	27	58.7	65.9	27.5	38.6	47.7
Mean		45.0	67.6	58.8	40.2	52.9

TABLE 4.—*Significant mean differences between sugar-metabolyzing enzymes from leaves of immature sugarcane sprayed with varying levels of IAA, 2,4-D, and MH*[1]

Item	Days after treatment	Control	IAA	2,4-D	MH
1, β-Glycerophosphatase	1				
	3		2 > 1*		
			2 > 3*		
			2 > 4*		
	9	1 > 2*			4 > 1*
		1 > 3*			4 > 2*
					4 > 3*
	27		2 > 4*	3 > 4*	
				3 > 1*	
2, UDPG-phosphatase	1		2 > 3*	3 > 4*	3 > 4
				3 > 1*	
	3		2 > 4*		
	9				
	27		2 > 4*	3 > 4*	
			2 > 1*		
3, ATP-ase	1				
	3		2 > 1**		
			2 > 3**		
			2 > 4**		
	9				4 > 2*
					4 > 1*
					4 > 3**
	27		2 > 4*	3 > 4**	
			2 > 1*	3 > 1**	
4, Glucose-1-phosphatase	1		2 > 3*		2 > 4*
	3	1 > 4*	2 > 3*	3 > 4*	
			2 > 1**		
			2 > 4**		
	9	1 > 3*	2 > 3**		
		1 > 4*	2 > 4**		
	27				
5, Glucose-6-phosphatase	1				
	3		2 > 3**		
			2 > 1**		
			2 > 4**		
	9	1 > 4**			
		1 > 2**			
		1 > 3**			
	27				

TABLE 4.—*Continued*

Item	Days after treatment	Control	IAA	2,4-D	MH
6, Fructose-6-phosphatase	1		2 > 1*		
			2 > 3*		
			2 > 4*		
	3		2 > 3**		
			2 > 1**		
			2 > 4**		
	9				
	27	1 > 4**	2 > 4**	3 > 4**	
7, Fructose-1,6-diphosphatase	1	1 > 3**	2 > 3*		
			2 > 4*		
	3		2 > 3		
			2 > 1		
			2 > 4		
	9		2 > 1**	3 > 1**	4 > 1**
	27		2 > 4*	3 > 4*	
8, 3-PGA phosphatase	1		2 > 4*		
	3		2 > 1**		
			2 > 3**		
			2 > 4**		
	9		2 > 1*		4 > 1*
			2 > 3*		4 > 3*
	27		2 > 1**	3 > 1**	
			2 > 4**	3 > 4**	
9, Hexokinase	1		2 > 3*		
			2 > 1**		
			2 > 4*		
	3				
	9				
	27				4 > 1**
					4 > 3**
					4 > 2**
10, Invertase	1	1 > 4*	2 > 4*	3 > 4*	
	3				
	9				
	27	1 > 3*	2 > 3*		4 > 2*
					4 > 3*
11, Amylase	1	1 > 4*	2 > 1**	3 > 4*	
			2 > 3**		
			2 > 4**		
	3				
	9	1 > 3**	2 > 4**		
		1 > 4*	2 > 3**		
	27		2 > 3**		
			2 > 1**		
			2 > 4**		

TABLE 4.—*Continued*

Item	Days after treatment	Control	IAA	2,4-D	MH
12, Aldolase	1				
	3				
	9	1 > 4*	2 > 4*		
	27	1 > 3*	2 > 3*		
		1 > 4**	2 > 4**		
13, Peroxidase	1		2 > 1**		
			2 > 3**		
			2 > 4**		
	3				
	9		2 > 3*		
			2 > 1*		
			2 > 4*		
	27		2 > 3*	3 > 4*	
			2 > 4**	3 > 1*	
			2 > 1**		
14, Isocitric acid dehydrogenase	1		2 > 1*		
			2 > 3**		
			2 > 4**		
	3		2 > 3*		
			2 > 1*		
			2 > 4**		
	9	1 > 3*	2 > 3*		
	27		2 > 3**		
			2 > 4**		
			2 > 1*		
15. Triose phosphate dehydrogenase	1	1 > 2*		3 > 2*	4 > 2**
	3		2 > 1*	3 > 1*	4 > 1*
	9	1 > 2*			
		1 > 3*			
	27				
16, Transaminase	1		2 > 3*		
			2 > 1*		
			2 > 4*		
	3				
	9		2 > 1**	3 > 1**	4 > 1*
	27		2 > 4**	3 > 4**	
			2 > 1*	3 > 1*	
17, Phosphohexose isomerase	1	1 > 4**	2 > 4**	3 > 4**	
	3			3 > 4**	
				3 > 1**	
				3 > 2**	

TABLE 4.—*Concluded*

Item	Days after treatment	Control	IAA	2,4-D	MH
	9		2 > 3* 2 > 1* 2 > 4*		
	27	1 > 3**	2 > 3**		4 > 2** 4 > 1** 4 > 3**
18, Phosphoglyceryl kinase	1				
	3	1 > 4*	2 > 1** 2 > 4** 2 > 3**	1 > 3*	
	9	1 > 3** 1 > 4**	2 > 1* 2 > 3** 2 > 4**		
	27				3 > 1** 3 > 2** 3 > 4**
19, Condensing enzyme	1	1 > 2*		3 > 2*	4 > 1* 4 > 2**
	3	1 > 3*			
	9		2 > 1* 2 > 3*		4 > 2** 4 > 1** 4 > 3**
	27				
20, Glucose oxidase	1				
	3	1 > 4*	2 > 3* 2 > 4*		
	9		2 > 4* 2 > 1**	3 > 1**	
	27	1 > 4** 1 > 3**	2 > 4** 2 > 3**		4 > 3*
21, Catalase	1	1 > 4**	2 > 3** 2 > 1** 2 > 4**	3 > 1** 3 > 4**	
	3				
	9	1 > 4** 1 > 2**		3 > 1** 3 > 4** 3 > 2**	4 > 2*
	27	1 > 4*	2 > 1* 2 > 4**	3 > 1* 3 > 4**	

[1] The numbers and symbols used in table 4 have the following meanings: Nos. 1, 2, 3, and 4 refer to mean values for control, IAA, 2,4-D, and MH treatments, respectively. The notation 1 > 4** signifies that the mean value for the control treatment was larger than the mean for the MH treatment, and that the difference was significant at the 1-percent level.

periments (fig. 6), although this time the sucrose fluctuations were brought about by totally different and presumably unrelated treatments.

Transaminase

Transaminase was stimulated most effectively by IAA (fig. 7). This constitutes one of the most reasonable enzyme responses recorded during

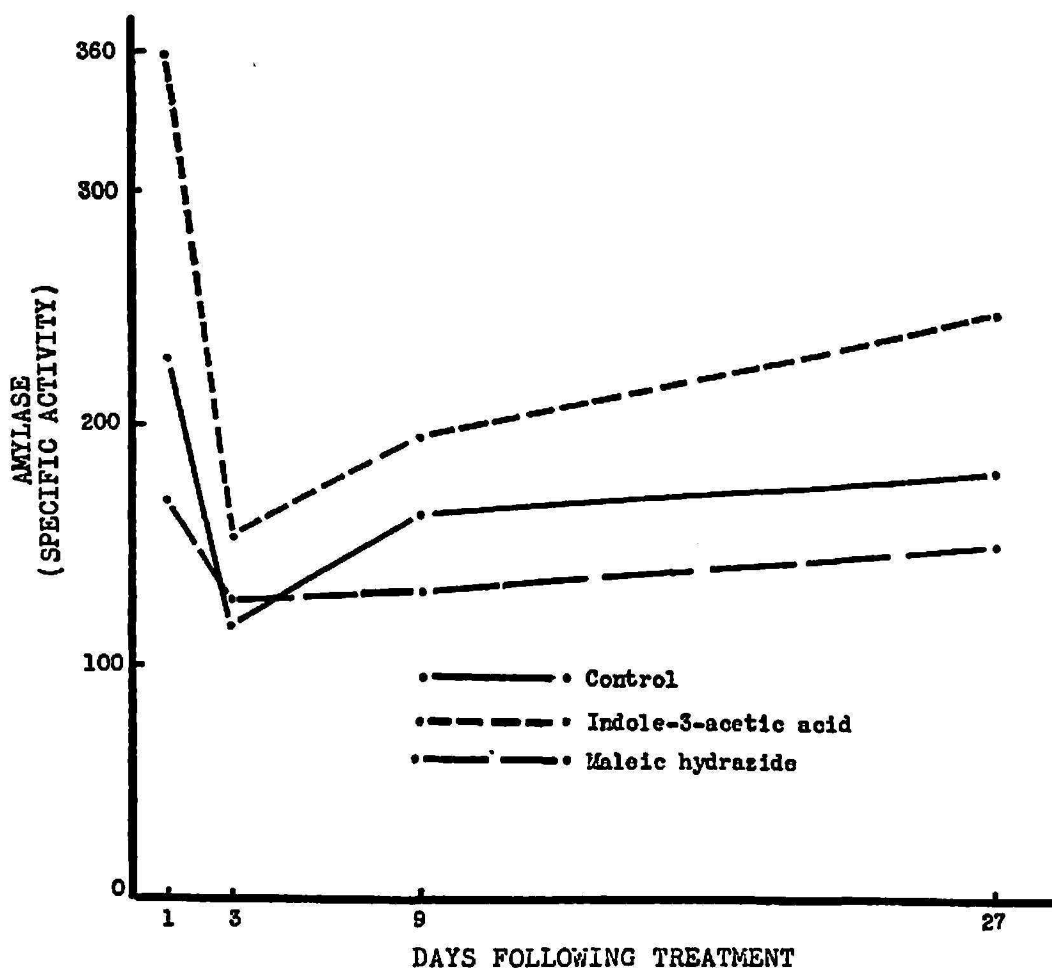


FIG. 3.—Activity of the enzyme amylase from leaves of immature sugarcane following spray application of indole-3-acetic acid and maleic hydrazide.

the study, since the growth responses traditionally attributed to IAA must require an increased formation of amino acids, for protein synthesis, from the organic acids derived via carbohydrate metabolism. A less pronounced stimulation of transaminase was affected by 2,4-D.

Phosphohexose Isomerase

As our enzyme assays progressed it became apparent that the length of time following treatment was nearly as important as the properties of the growth regulator itself in determining its effect upon enzyme action. This

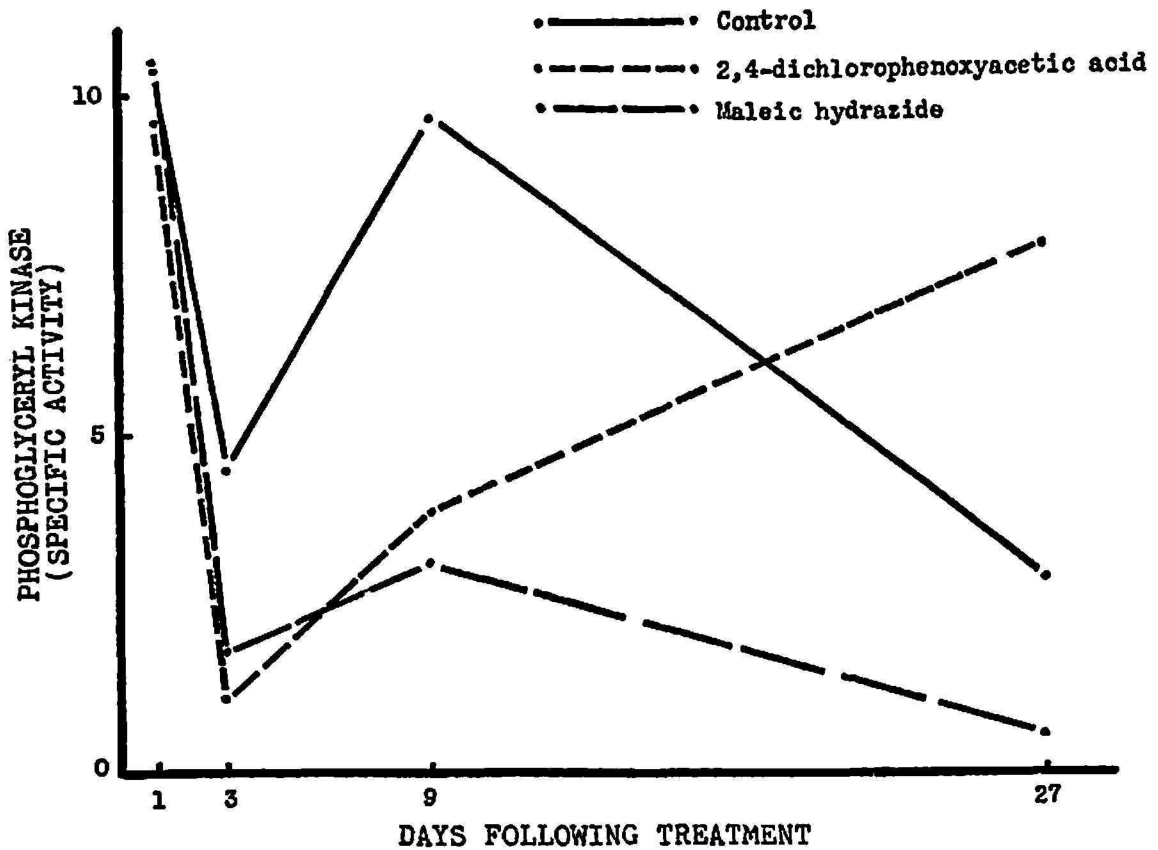


FIG. 4.—Action of the enzyme phosphoglyceryl kinase from leaves of immature sugarcane following spray application of 2,4-dichlorophenoxyacetic acid and maleic hydrazide.

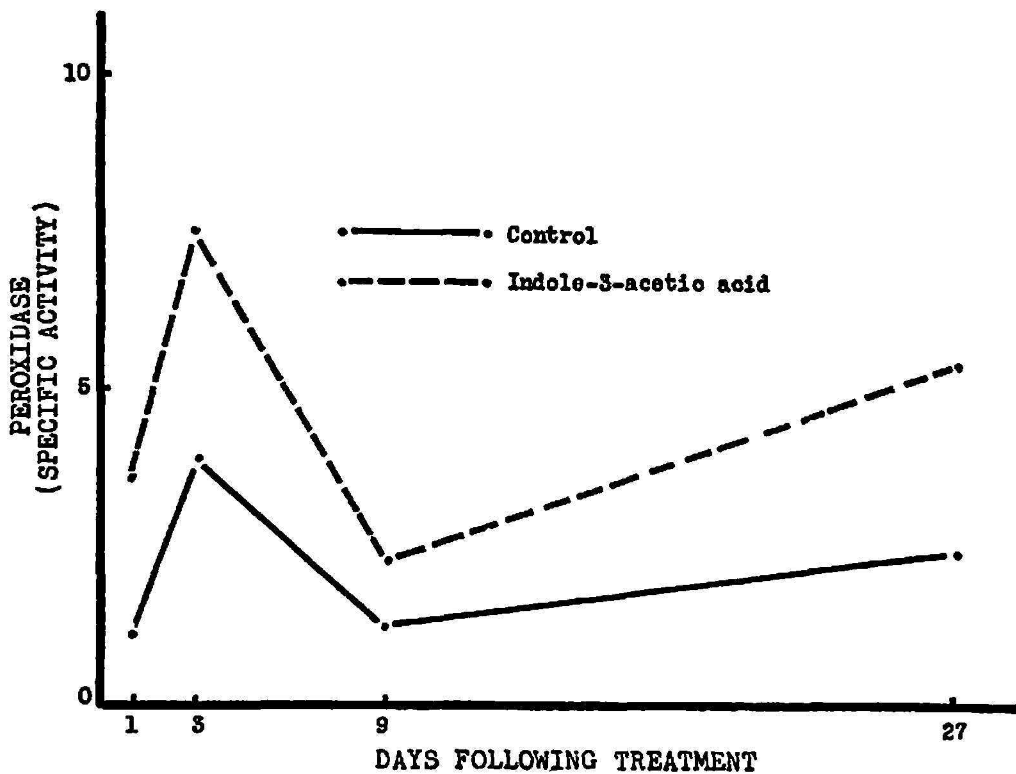


FIG. 5.—Action of the enzyme peroxidase from leaves of immature sugarcane following spray application of indole-3-acetic acid.

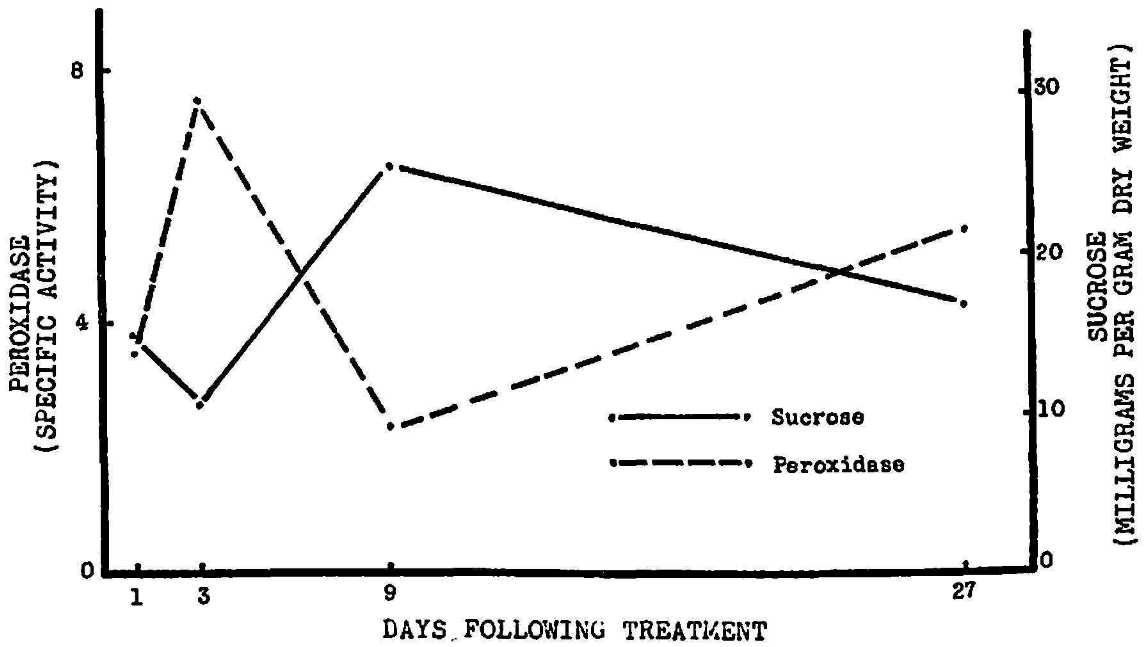


FIG. 6.—Inverse relationship between the sucrose content and peroxidase activity of leaves from immature sugarcane following spray application of indole-3-acetic acid.

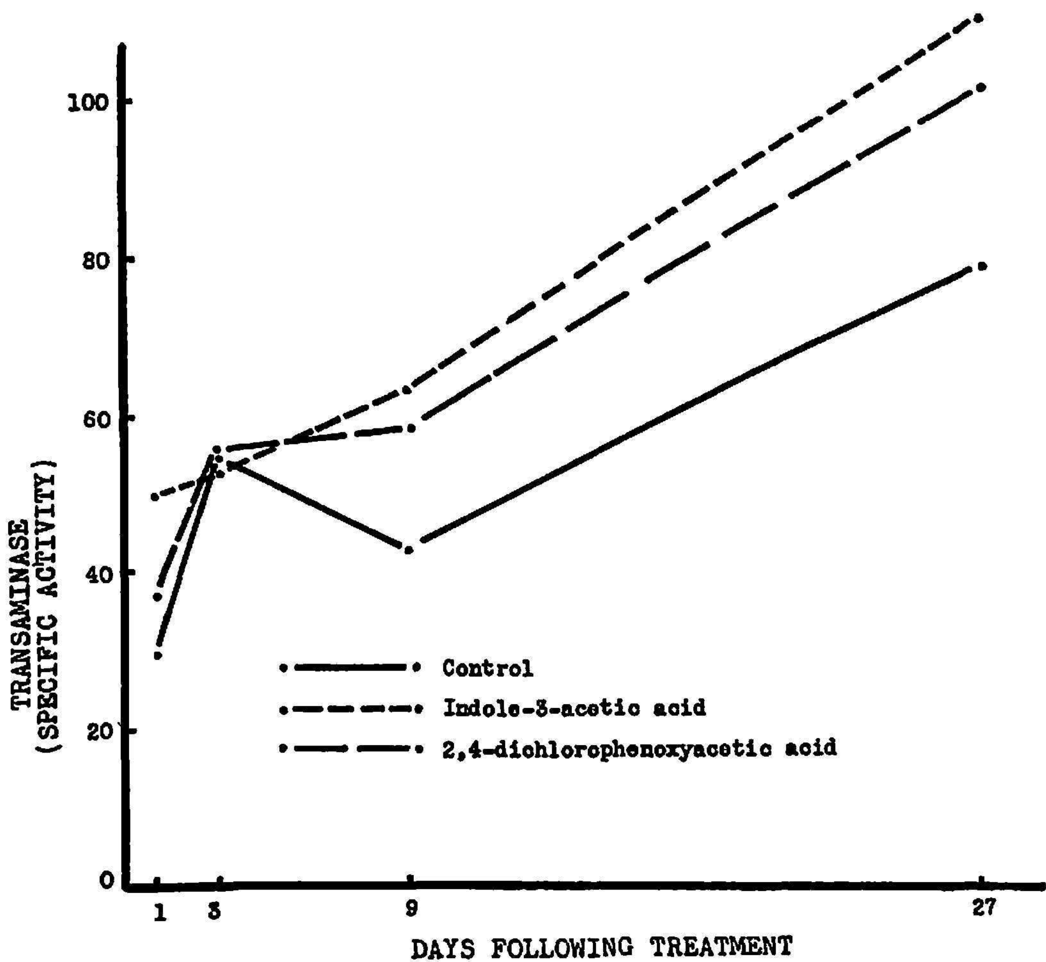


FIG. 7.—Effects of indole-3-acetic acid and 2,4-dichlorophenoxyacetic acid on the transaminase enzyme from leaves of immature sugarcane.

principle is vividly illustrated by the relationships of MH with the enzyme phosphohexose isomerase (fig. 8). Suppressed by MH to less than one-sixth of control values within 1 day of treatment, the enzyme increased some eighteenfold by the end of the study when its action level was more than double that of controls.

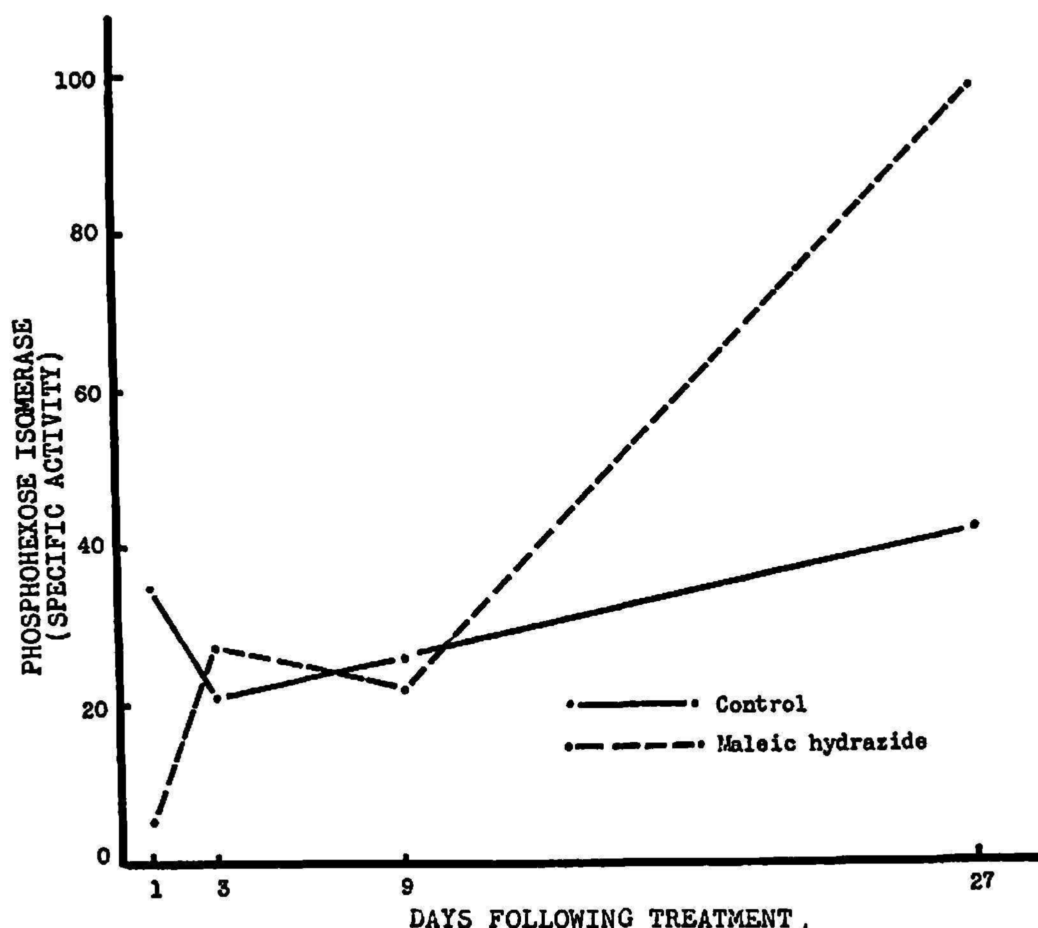


FIG. 8.—Effects of maleic hydrazide on the enzyme phosphohexose isomerase from leaves of immature sugarcane.

Condensing Enzyme

The condensing enzyme represents one of the few systems which IAA failed to stimulate, and, in fact, the enzyme was suppressed by IAA to about 25 percent of control activity within 1 day following treatment (fig. 9). Little IAA effect upon this system was recorded during the remainder of the study. Maleic hydrazide strongly stimulated the enzyme among samples taken 1 and 9 days following treatment.

DISCUSSION

ACID PHOSPHATASES *vs.* SUCROSE CONTENT

The stimulation of acid phosphatases by IAA seemed to dispell our previous contention that high leaf-sucrose production required a suppres-

sion of the ATP and glucose-phosphate hydrolyzing enzymes. In other words, if phosphatase suppression was essential, how could IAA and 2,4-D have promoted sucrose while they were simultaneously stimulating phosphatases? Nevertheless the action curves for certain phosphatases seemed to indicate a close relationship between these enzymes and sucrose. The curve for β-glycerophosphatase, for example, reveals an almost exact inverse relationship with sucrose among plants treated with IAA (fig. 10). This led us to suspect that at least one phosphatase in our extracts was intimately involved in sucrose production, and that it must be suppressed

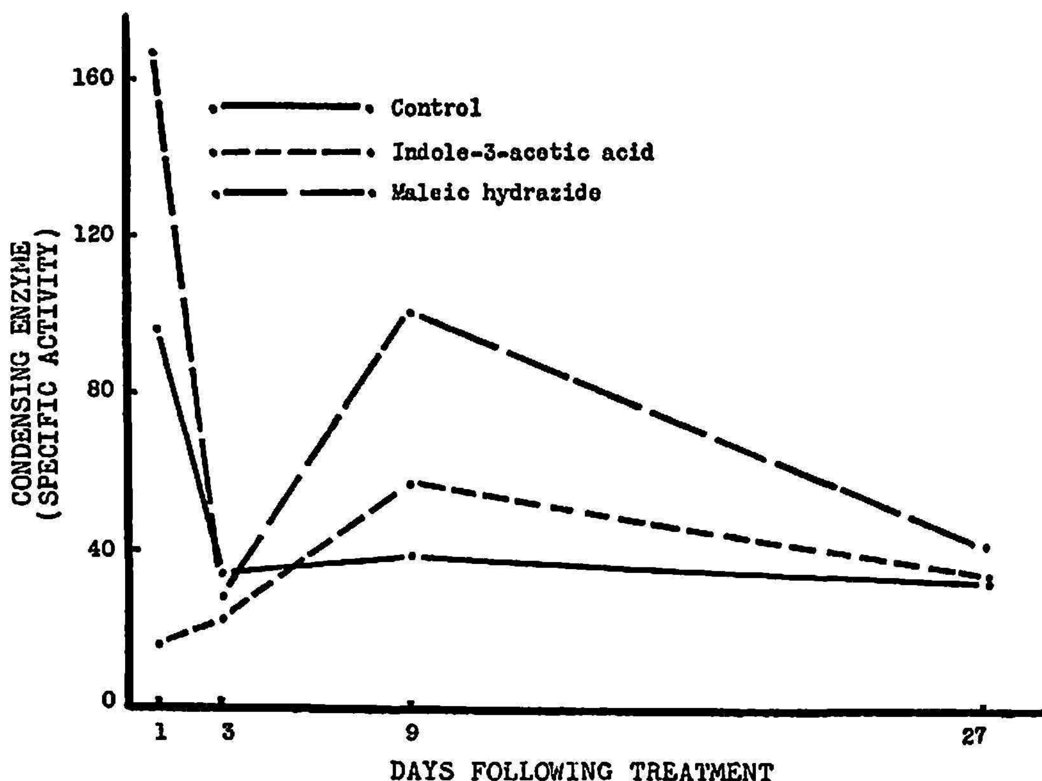


FIG. 9.—Effects of indole-3-acetic acid and maleic hydrazide on the condensing enzyme from leaves of immature sugarcane.

in order for sucrose to accumulate. The enzyme most nearly fitting this description was glucose-6-phosphatase (fig. 11), with ATP-ase answering as second choice (fig. 12). These also were apparently the most critical systems during our earlier work (*2*).

The typical phosphate hydrolysis reaction may be expressed as follows, with R representing the transferable sugar, glyceric acid, or adenylic acid group, and H_2O serving as the general acceptor (*19*):

$$R{-}O{-}PO_3H_2 + H_2O \rightarrow ROH + H_3PO_4$$

Leloir and coworkers (*12*) have developed the following series of reactions involving hexose and uridine phosphates in the biosynthesis of sucrose:

1, Uridine triphosphate (UTP) + Glucose-1-phosphate →
Uridine diphosphate glucose (UDPG) + Pyrophosphate (PP)

2, UDPG + Fructose → Sucrose + Uridine diphosphate (UDP)

3, UDPG + Fructose-6-phosphate (F-6-P) → Sucrose phosphate + UDP

During the absence or suppression of glucose-6-phosphatase, the glucose phosphate could become available for sucrose synthesis by either of the

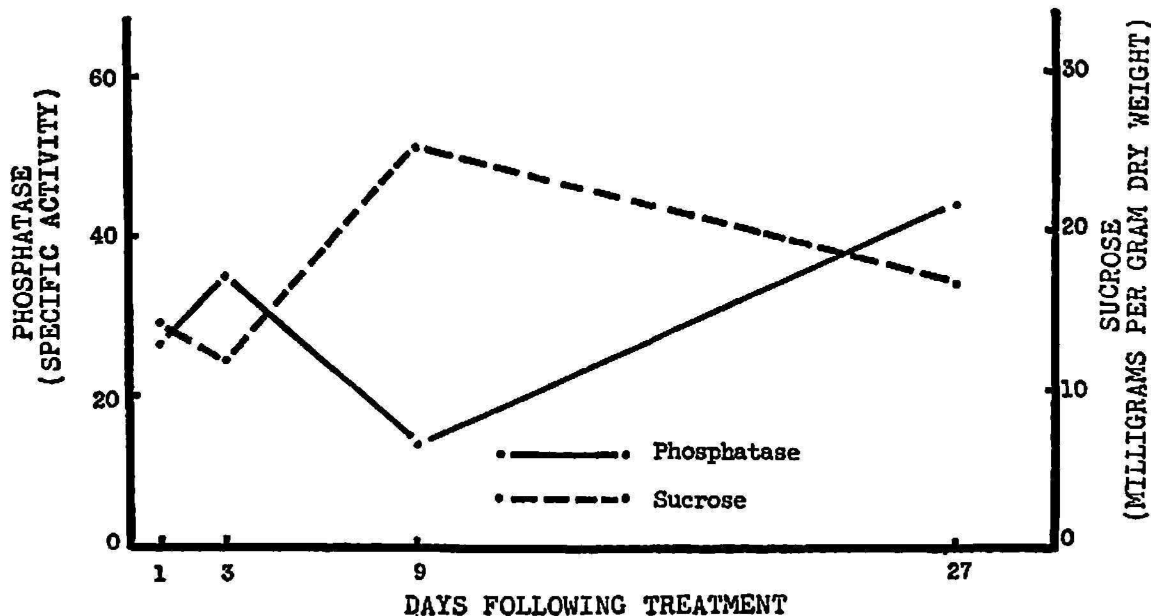


Fig. 10.—Inverse relationship between sucrose content and acid phosphatase of leaves from immature sugarcane following spray application of indole-3-acetic acid.

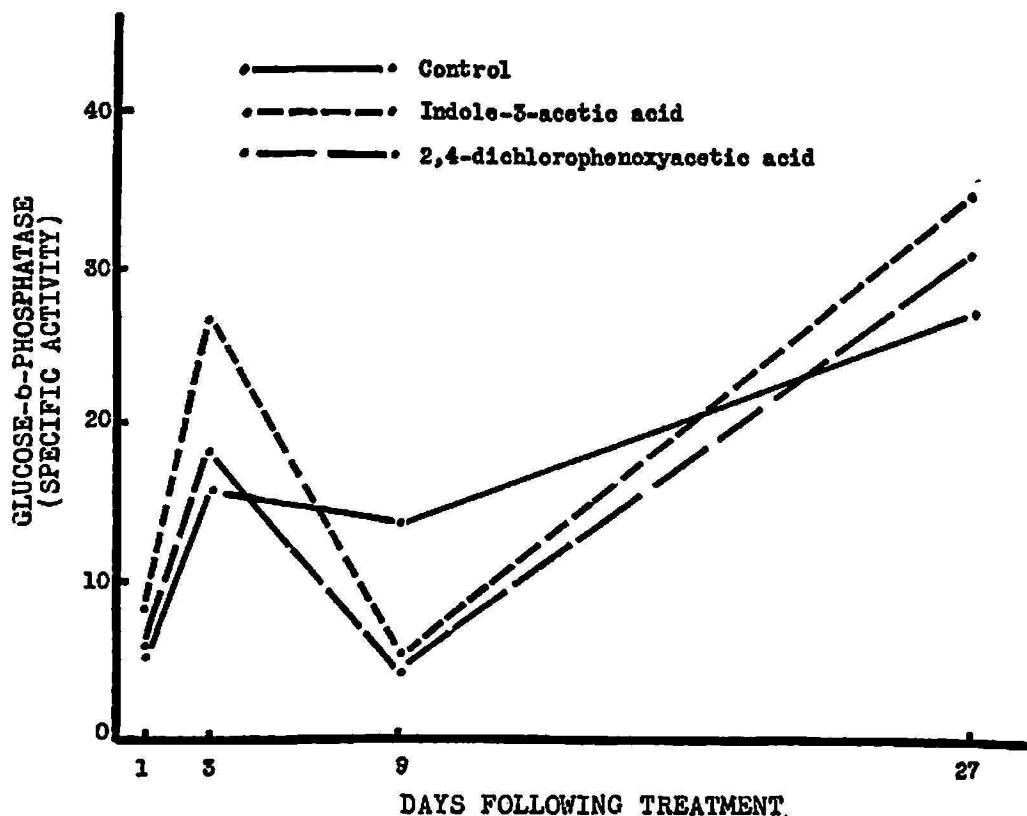
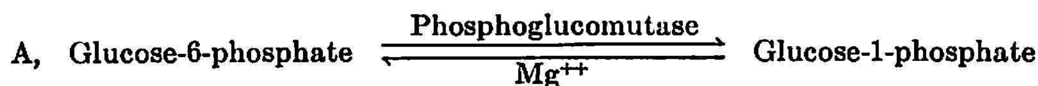


Fig. 11.—Action of glucose-6-phosphatase from leaves of immature sugarcane following spray application of indole-3-acetic acid and 2,4-dichlorophenoxyacetic acid.

following two enzymatic reactions (*19*):



A, Glucose-6-phosphate $\underset{Mg^{++}}{\xrightleftharpoons{\text{Phosphoglucomutase}}}$ Glucose-1-phosphate

B, Glucose-6-phosphate $\xrightleftharpoons{\text{Phosphohexoisomerase}}$ Fructose-6-phosphate

In the presence of an active glucose-6-phosphatase, however, the supply of glucose phosphate would be converted to free glucose plus inorganic

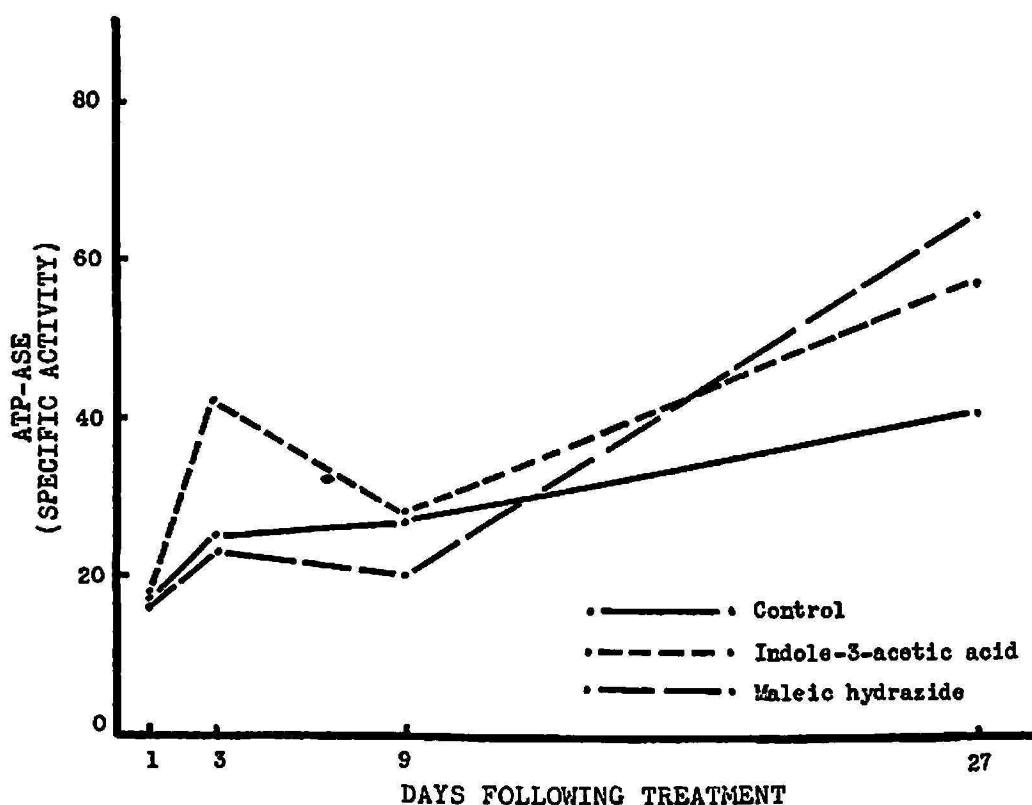


FIG. 12.—Action of the ATP-hydrolyzing enzyme from leaves of immature sugarcane following spray application of indole-3-acetic acid and 2,4-dichlorophenoxy-acetic acid.

phosphate, effectively blocking both of the above reactions. In all probability this was an important factor in establishing the inverse relationship observed between glucose-6-phosphatase and the sucrose levels in this study.

The potential importance of the ATP-hydrolyzing system in cane-leaf tissue has been thoroughly discussed in a previous paper (*2*). Another aspect of the ATP-ase reaction which has been pointed up by the IAA treatment of this study requires clarification. Figure 12 indicates that the overall shape of the ATP-ase curve closely resembles that of glucose-6-phosphatase, or, in other words, the enzyme exhibited a definite decline when sucrose was high and achieved a high activity level when sucrose was low. However,

the absolute value for ATP-ase at the IAA period of high sucrose was no lower than that of the control plants, which themselves revealed no such accumulations of sucrose (table 5).

If this suppressed level of ATP-ase was necessary for sucrose production, why didn't the control plants also respond with increased sucrose? The obvious answer lies in the proposition that IAA had literally lifted the treated plants to a new metabolic level, at which, we assume, they were capable of both synthesizing and metabolizing sucrose at a more rapid rate than control plants. Thus the mean ATP-ase specific activity value of 26 signifies a definite suppression among IAA plants, but represents nothing unusual for control plants. We should remember that the enzyme's potential is a relative factor, more logically based upon the overall physiological state of the plant than upon any scale of absolute values we can devise. It is also apparent that comparisons with control plants for any other criteria than treatment effects can be highly misleading.

The capability of IAA- and 2,4-D-treated plants temporarily to increase sucrose production (table 5) cannot be attributed to a direct suppressing effect upon phosphatase by these compounds. Our data tell us that IAA stimulated phosphatases in general, and that the one period when sucrose made significant increases was accompanied by a relative decline of several specific phosphatases. This is further evidence that the acid phosphatases can affect sucrose biosynthesis, and that some degree of suppression of these systems is necessary for maximum sucrose production. But IAA acted as a phosphatase stimulant and, because it can promote the depletion of phosphorylated metabolites, IAA will not likely serve as a means of effecting long-term sucrose increases in sugarcane. Maleic hydrazide, on the other hand, was able to suppress a number of phosphatases and stimulated none, and for this reason we feel that MH has greater potential as an agent for promoting sucrose production, in spite of the fact that it brought about no spectacular increases during this study.

IAA STIMULATION OF CARBOHYDRATE-METABOLIZING ENZYMES

In addition to the acid phosphatases, IAA greatly increased the action of other enzymes involved in carbohydrate metabolism, including amylase, hexokinase, transaminase, and peroxidase. The presence of a powerful amylase system in cane leaves has already led us to suggest that starch may serve as a glucose reserve for both hexose metabolism and sucrose synthesis (*2*), with glucose being released through the combined action of amylase and maltase systems. The growth-promoting properties of IAA must logically increase the plant's need for energy and organic acid reserves which are derived from the increased metabolism of glucose. The immediate source of the needed glucose may well be starch, as evidenced by the considerable stimulation of amylase within 24 hours of IAA treatment. With

TABLE 5.—*Significant mean differences between leaf sugars of immature sugarcane supplied with varying levels of indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and maleic hydrazide (MH)*[1]

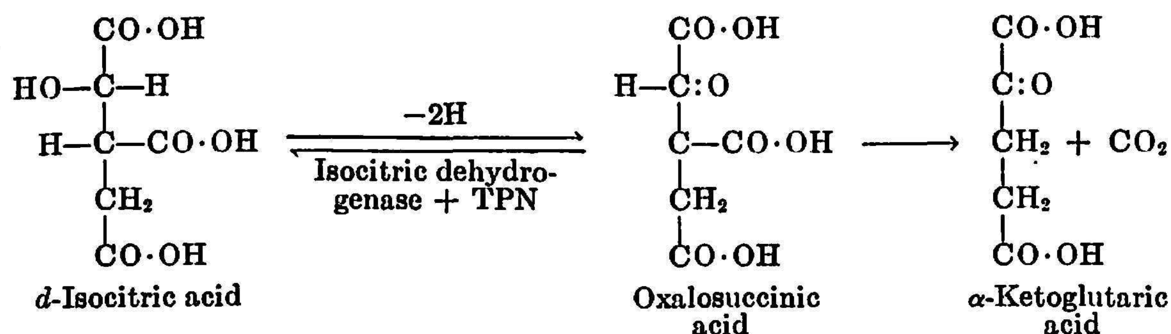
Sugar	Days following treatment	Control	IAA	2,4-D	MH
Total ketose	1	1 > 3*	2 > 3*		4 > 3*
	3		2 > 3*		
			2 > 1*		
			2 > 4*		
	9			3 > 1*	
	27	1 > 3*	2 > 3*		4 > 3**
Sucrose	1				
	3			3 > 1*	
				3 > 2*	
				3 > 4*	
	9		2 > 4*	3 > 4**	
			2 > 1*	3 > 1**	
	27	1 > 3*	2 > 3*		4 > 3*
Fructose	1				
	3				
	9				
	27			3 > 1*	
				3 > 2*	
Total reducing	1				
	3		2 > 4**		
			2 > 3**		
			2 > 1**		
	9				
	27				4 > 2**
					4 > 3**
					4 > 1**
Glucose	1				
	3			3 > 1*	
				3 > 2*	
	9				
	27		2 > 3*		4 > 2**
					4 > 1**
					4 > 3**

[1] The numbers and symbols used in table 5 have the following meanings: Numbers 1, 2, 3, and 4 refer to mean values for control, IAA, 2,4-D, and MH treatments, respectively. The notation 1 > 4** signifies that the mean value for the control treatment was larger than the mean for the MH treatment, and that the difference was significant at the 1-percent level.

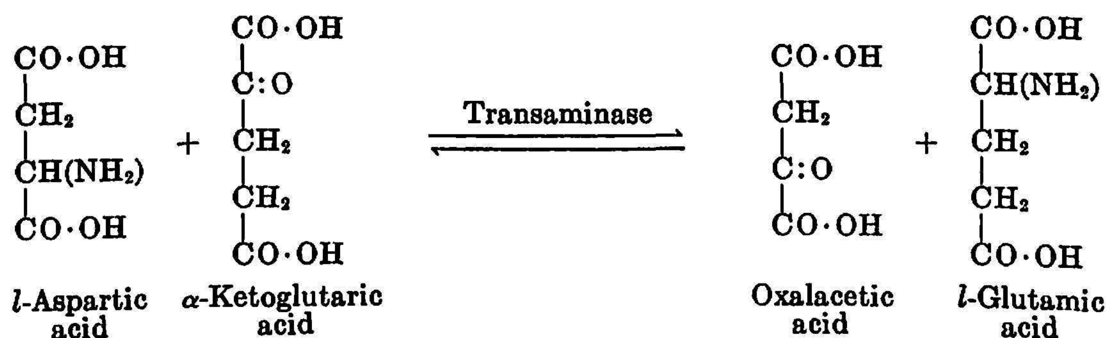
the aid of maltase, this would provide the plant with a vastly increased supply of glucose.

It is interesting to note that the early IAA stimulation of amylase was accompanied by a threefold increase in hexokinase, providing a means for converting the newly released glucose into glucose-6-phosphate. The phosphorylated glucose would then be able to enter sucrose biosynthesis by the enzymatic reactions A and B, or enter into the glycolytic pathway with the ultimate production of ATP and pyruvate. That much of the hexose was in fact metabolized through glycolysis is suggested by the early increase in transaminase, the enzyme which would play a major role in converting the organic acid products of glycolysis into the amino acid building units needed for protein synthesis. It should also be noted that the initially high activity of amylase and hexokinase soon returned to near-control levels, suggesting perhaps that the initial demand for additional glucose had been satisfied. Other sugar-metabolizing enzymes such as phosphohexose isomerase and aldolase eventually increased and transaminase remained generally high.

Additional insight into the relationship between isocitric dehydrogenase and transaminase was brought forth as a result of the IAA treatment (fig. 13). Isocitric dehydrogenase forms a critical link between carbohydrate degradation and protein synthesis (*1*) by catalyzing the initial step of the following reaction:



We used the transaminase assay of Tonhazy and coworkers (*25*) which employs an excess of *l*-aspartic and α-ketoglutaric acids, in the presence of enzyme, and the reaction rate is based upon the quantity of oxalacetic acid formed:



While it is evident that both isocitric dehydrogenase and transaminase deal with closely related compounds, the dehydrogenase is actually one of the last of the series of carbohydrate-metabolizing enzymes, and the transaminase one of the first of a series of protein-building systems. We see from figure 13 that both enzymes were initially stimulated by IAA, but that isocitric dehydrogenase soon exhibited a temporary decline. This again

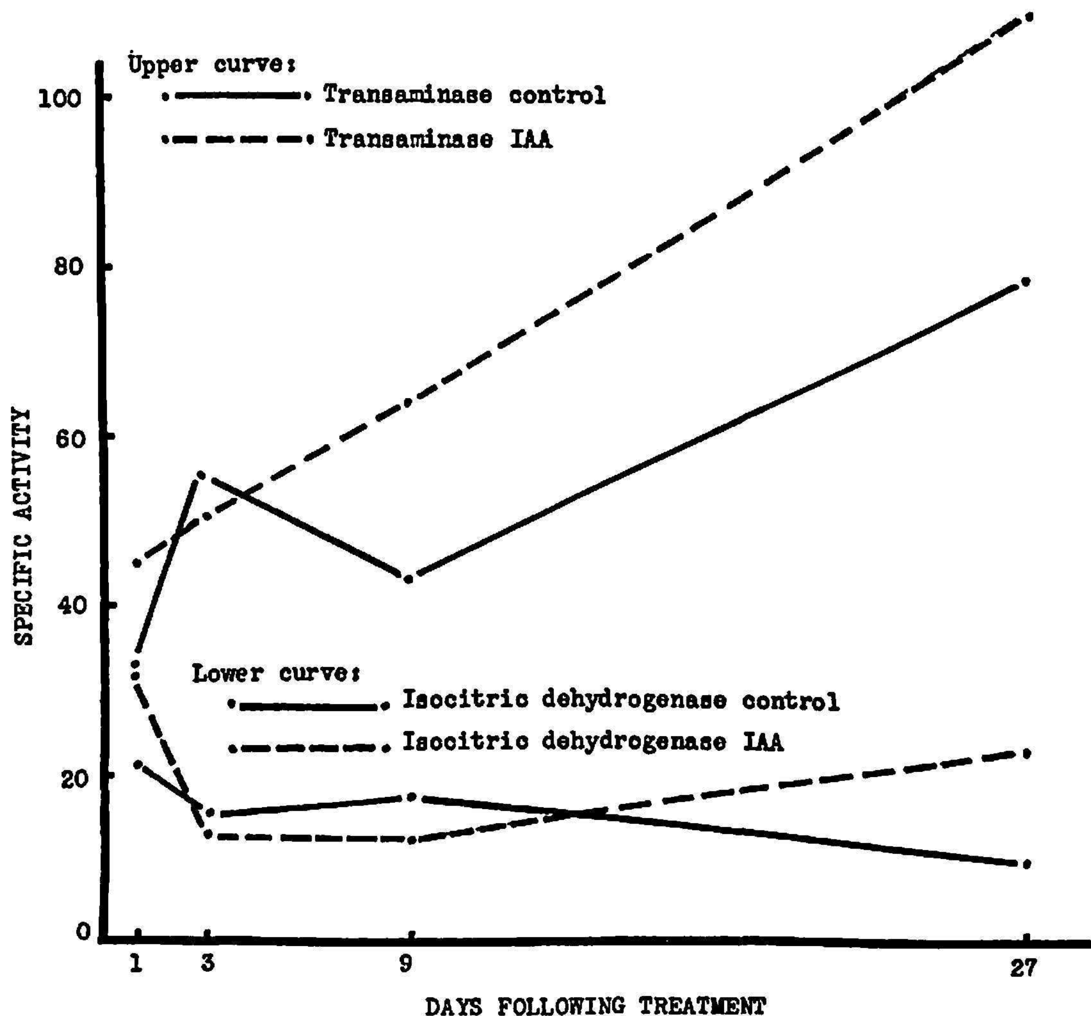


FIG. 13.—Comparison of isocitric dehydrogenase and transaminase from leaves of immature sugarcane following spray application of IAA.

was the same general response of the glucose phosphatases, amylase, invertase, hexokinase, and phosphohexose isomerase—each of which takes part in sugar metabolism, and the temporary decline of which appears to have permitted a short-lived increase of sucrose. Thus we find that while the two enzymes are closely associated as a team, each remains true to its own task in two distinct physiological processes, the one slackening its action in a carbohydrate-degradating sequence while the other continues increasingly active in its role as a synthesizing catalyst.

Our interest in peroxidase has been briefly mentioned because of the enzyme's apparent relationship with sucrose, and figure 6 illustrates the inverse relationship between peroxidase and sucrose brought forward by the IAA treatment. The possibility that this enzyme may serve as a terminal oxidase has already been put forward (*2*). The work herein reported again emphasizes the possibility of a key peroxidase role, since it was again related to sucrose content, in spite of the fact that sucrose had been altered by an entirely different type of treatment.

Perhaps because of the versatile ability of peroxidase to catalyze the oxidation of metabolites (*5*), this enzyme alone exemplifies nearly all of the enzyme growth-regulator relationships disclosed by this study. Its activity fluctuations varied closely with sucrose changes, it was greatly stimulated by IAA while still retaining the overall reaction curve of controls, it was greatly inhibited by MH, and it illustrates the importance of time by eventually recovering from the almost complete suppression by MH.

PHYSIOLOGICAL BASIS FOR THE SUPPRESSION AND STIMULATION OF ENZYMES BY GROWTH REGULATORS

When explaining the mechanism of enzyme suppression by any compound it is tempting to bring forth the concepts of competitive and noncompetitive inhibition. In a few instances the depressing effect of MH on cane-leaf enzymes does answer closely to the expected action of a competitive inhibitor. Competitive inhibition depends upon a lack of specificity for the reaction on the active enzyme site (*19*). Because of this the enzyme may "mistake" an inhibitor molecule for its true substrate, and, in fact, the inhibitor often is structurally related to the true substrate. The reaction site is then blocked to the substrate. A second characteristic of competitive inhibition is its reversibility, or in other words, the inhibitor may be displaced by the true substrate when enough substrate molecules become available effectively to compete with the inhibitor. A common test for competitive inhibition is to measure the degree of inhibition removal as the concentration of true substrate is gradually increased. In our work the suppression of phosphohexose isomerase and peroxidase by MH was possibly effected by this kind of inhibition, since in both instances the enzymes later experienced complete recovery from the suppression.

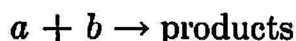
More difficult to explain is the suppression of aldolase by MH. The effect was not detected until 9 days following treatment and was not really severe until 27 days afterward. There was but one MH treatment, so we know that the material which caused enzyme suppression at 27 days must also have been within the plant at 1 and 3 days, when no effect was recorded. The enzyme may have been protected temporarily by endogenous compounds capable of reacting with the MH, or possibly the MH was able to interfere

with the enzyme only after a new activation energy level had been acquired by the substrate.

A basic feature of noncompetitive inhibition is its nonreversibility. The inhibitor apparently combines with the enzyme at some site other than the usual point of substrate attachment, and from there it permanently blocks the reactive site in spite of substrate concentration (*19*). The suppression of glucose-6-phosphatase by MH, which was moderate but consistent through all sampling periods, was possibly attributable to noncompetitive inhibition.

The most difficult enzyme-regulator responses to explain are those in which a stimulation or inhibition of the enzyme at one period is followed by a reversal of this effect at a later harvest. No matter what basis we use to explain one response, some other mechanism must be brought forth to explain its reversal, and this approach is not well substantiated by our present understanding of enzyme inhibitor and activator relationships. Nevertheless the very great number of such reversible responses brought out by these experiments leads us to believe that a single, common basis exists which fits well with the physiological needs and capabilities of the plant.

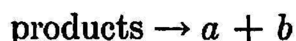
To the investigator confronted with explaining a reversible regulator effect upon an enzyme, as for example the above-mentioned MH-isomerase relationship (fig. 8), a reasonable approach is to consider the effects of the regulator upon the activation energy level of the substrate in question. It is generally believed that only those molecules of a reactant which possess a certain minimum energy, or "activation energy", are able to enter into reactions which will rearrange its electronic structure and leave it in an altered state. This minimal energy level is often regarded as an energy barrier which the molecule must surmount before it can enter reactions, and enzymes are able to increase reaction rates by lowering activation energy levels (*5*). Thus the reaction



$$a + b \rightarrow \text{products}$$

is dependent on the acquisition of a minimal energy increment (E_1*), which can be achieved far more rapidly in the presence of enzyme.

Eyring (*9*) greatly increased our understanding of the activation energy concept (fig. 14) by proposing that a second energy increment (E_2*) is required for the reverse reaction:



$$\text{products} \rightarrow a + b$$

Since the forward reaction is dependent upon the magnitude of E_1*, and the reverse reaction upon E_2*, the energy change is equivalent to $E = E_1^* - E_2^*$, and, therefore, the knowledge of ΔH (standard heat-energy

change) and ΔF (standard free-energy change) cannot be used for the calculation of reaction rate (*10*, p. 265). However, the concepts of E_1* and E_2* lend themselves readily to the explanation of biological phenomena such as enzyme stimulation and inhibition. Assuming that such compounds as IAA and MH form enzyme-regulator complexes after entering the plant, a regulator capable of lowering E_1* would, in effect, serve as an enzyme activator by shifting the reaction equilibrium ($a + b \rightleftharpoons$ products) to the right. This could well explain the apparent capability of IAA to stimulate

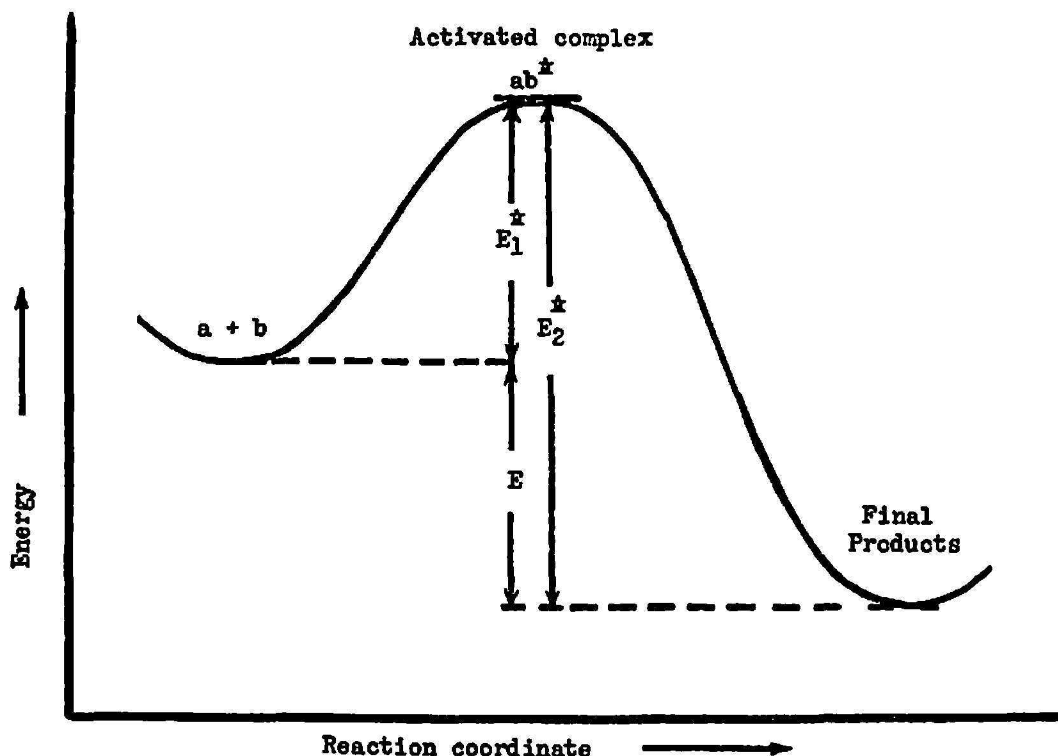


FIG. 14.—Schematic formulation of the mechanism of a reaction, according to Eyring.

rather than retard reactions. On the other hand, a compound exerting its effect by lowering E_2* would promote the reverse reaction, shift the equilibrium to the left, and, in effect, serve as an inhibitor. Such a preference for the E_2* effect would explain the enzyme-suppressing action of MH. It must also be remembered that the relation between reaction velocity and activation energy is an exponential one (*5*, p. 8) and very slight changes in E_1* and E_2* could readily account for enzyme fluctuations of the magnitude we have recorded.

To place the regulator-activation energy relationships in better perspective, we should remember that the regulators were supplied only once, and this at a time when the plants had just entered their grand period of growth. The growth-regulating materials must have formed most of the possible

complexes with the respective enzymes within the 24 hours which passed before the initial harvest was taken. Consequently we must have been dealing with a series of new enzyme-regulator equilibria, at least partly established by the time we took our first samples. Each equilibrium would then have been subject to change by breakdown or metabolism of the regulator with time, the dilution of the regulator via translocation to newly forming tissues, dilution by reactions with newly produced endogenous metabolites, and by any changing physiological demands made by the plant upon its enzymes as it grew older. With the hypothesis that IAA, 2,4-D, and MH were affecting E_1^* and E_2^* levels, the enzyme changes observed during this study appear not only possible and logical, but it would have been most unexpected had many such enzyme changes had not occurred with the passage of time.

SUMMARY AND CONCLUSIONS

Indole-3-acetic acid, 2,4-dichlorophenoxyacetic acid, and maleic hydrazide were applied as foliar sprays to 10-week-old sugarcane plants during initial studies of the interrelationships of growth-regulating materials with the sugar-metabolizing enzymes of sugarcane. Leaf samples were harvested at 1, 3, 9, and 27 days following treatment for sugar and enzyme assays. Sugar analyses were run for total ketoses, sucrose, fructose, and total reducing sugars, with glucose being determined by calculation. A series of acid phosphatase assays were conducted using as substrates the following compounds: β-glycerophosphate, adenosinetriphosphate, uridine diphosphate glucose, glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, and 3-phosphoglyceric acid. Additional enzymes included invertase, amylase, hexokinase, phosphohexose isomerase, aldolase, triosephosphate dehydrogenase, phosphoglyceryl kinase, condensing enzyme, isocitric acid dehydrogenase, transaminase, peroxidase, and glucose oxidase. All enzyme preparations consisted of dialyzed water-soluble protein extracted from freeze-dried leaf tissue and precipitated with ammonium sulfate between 35 and 95 percent of saturation.

The following results were obtained:

1. Highest sucrose levels were recorded 9 days after treatment among the IAA and 2,4-D groups. Maleic hydrazide had little effect on sucrose content.

2. Fructose content was highest at 9 days and declined sharply thereafter, indicating that fructose metabolism was a factor of primary importance. No relationship was evident between sucrose and reducing-sugar levels. Glucose-oxidase activity was relatively high at the period of greatest

sucrose production, suggesting a possible suppression of glycolysis by this system.

3. IAA generally stimulated enzyme activity while MH suppressed the enzymes or had no effect at all; 2,4-D effects were less pronounced. Enzyme stimulation or inhibition usually occurred within 1 day of treatment, though in several instances such effects were not recorded until 9 or 27 days.

4. High sucrose production at 9 days was accompanied by a pronounced decline of glucose-6-phosphatase, and to a lesser degree a decline of β-glycerophosphatase. The importance of retarded phosphatases in sucrose production is discussed.

5. IAA caused striking increases in amylase, hexokinase, and transaminase activity within 1 day following treatment. This indicates that starch may have served as a source of glucose to be metabolized as a part of the IAA growth-promoting action.

6. The general stimulation of acid phosphatases by IAA appeared to eliminate this material as an agent for long-term sucrose promotion. MH exhibited more suitable properties for this role by either retarding acid phosphatases or not affecting them at all.

7. Peroxidase was greatly stimulated by IAA, but the enzyme declined markedly when sucrose production was high. The inverse relationship of peroxidase with sucrose agrees with earlier observations of the same response.

8. The stimulating and inhibiting effects of the growth regulators upon enzymes are discussed on the basis of competitive and noncompetitive inhibition, and from the standpoint of altered activation energy level.

RESUMEN Y CONCLUSIONES

A plantas de caña de azúcar de 10 semanas de edad se les aplicó ácido acético-indole-3, ácido diclorofenoxyacético 2,4, e hidracida maleica, en aspersiones foliares, durante una serie de experimentos iniciales en los que se estudiaron las interrelaciones de los agentes que regulan el crecimiento, con las enzimas metabolizadoras del azúcar en la caña de azúcar. Se tomaron muestras de las hojas 1, 3, 9 y 27 días después del tratamiento a fin de hacer análisis para azúcar y enzimas. Se hicieron análisis para quetosas, sacarosa, fructosa y el total de azúcares reductores, en tanto que la glucosa se determinó a base de cálculos. Se efectuó una serie de análisis de fosfatasa ácida, usando los compuestos siguientes como sustratos:

β-glicerofosfato, adenocinetrifosfato, difosfato uridino de glucosa, fosfato de glucosa-1, fosfato de glucosa-6, fosfato de fructosa-6, fructosa-1, difosfato-6 y ácido fosfoglicério-3. Las enzimas adicionales incluían invertasa, amilasa, hexoquinasa, isomerasa fosfohexosa, aldolasa, dehidrogenasa

triosefosfatada, quinasa fosfogliceril, enzima condensadora, dehidrogenasa de ácido isocítrico, transaminasa, peroxidasa y oxidasa glucosada. Todas las preparaciones enzimáticas consistieron de proteína dializada, soluble en agua, extraída de tejido foliar secado por congelación y precipitada con sulfato amónico de un 35 a un 95 por ciento de saturación.

Se obtuvieron los resultados siguientes:

1. Los niveles más altos de sacarosa lográronse 9 días después del tratamiento, entre los grupos IAA y 2,4-D. La hidracida maleica tuvo muy poco efecto sobre el contenido de sacarosa.

2. El contenido de fructosa fue más alto a los 9 días y bajó drásticamente después, indicando que el metabolismo de la fructosa es un factor de importancia primaria. No hubo evidencia alguna de relación entre los niveles de sacarosa y los de azúcares reductores. La actividad de la oxidasa glucosada fue relativamente alta en el momento de mayor producción de sacarosa, lo cual sugiere una posible supresión de glicólosis mediante este procedimiento.

3. El IAA estimuló generalmente la actividad enzimática, mientras que el MH contuvo las enzimas o no surtió efecto alguno. Los efectos del 2,4-D fueron menos pronunciados. El estímulo o la inhibición enzimática tuvo lugar, usualmente, 1 día después del tratamiento, aunque hubo ocasiones en que estos efectos no se observaron hasta 9 ó 27 días después.

4. La alta producción de sacarosa a los 9 días se acompañó de una pronunciada reducción de fosfatasa glucosada-6, y, en menos grado, de β—glicerofosfatasa. Se discute la importancia de las fosfatasas retrasadas en la producción de sacarosa.

5. El IAA produjo aumentos notables en la actividad de la amilasa, hexoquinasa y transaminasa, durante el día que siguió al tratamiento. Esto indica que el almidón acaso sirvió como una fuente de glucosa que se metabolizaría a consecuencia de la acción estimulante del IAA.

6. El estímulo general de las fosfatasas ácidas por el IAA pareció eliminar este compuesto como agente estimulante a largo plazo para la producción de sacarosa. El MH evidenció tener propiedades más deseables para este propósito, retardando las fosfatasas ácidas, o no afectándolas en modo alguno.

7. El IAA estimuló grandemente la peroxidasa, pero la enzima se redujo marcadamente cuando la producción de sacarosa fue alta. La relación inversa de a peroxidasa con la sacarosa concuerda con observaciones previas hechas en condiciones en las cuales se obtuvieron resultados iguales.

8. Se discuten los efectos estimulantes e inhibitorios de los reguladores del crecimiento sobre las enzimas, a base de la inhibición competitiva y no competitiva, y desde el punto de vista de un nivel de energía de acción alterada.

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