Effects of Amylase and Invertase Regulators Upon Sugar Content, Protein Content, and Enzyme Activity of Immature Sugarcane

Alex G. Alexander[1]

INTRODUCTION

Although invertase and amylase rank among the most ancient of enzymes known to biologists (*15,18*),[2] little effort has been made to regulate these catalysts in living plants. With regard to sugarcane, it has become increasingly evident that their control might offer a means of promoting sugar formation. Studies in Puerto Rico have related both amylase (*1,2*) and invertase (*3,7*) to sucrose content. Hartt correlated activity with the areas of active sugar formation (*10*), and with potassium supply (*11,12*). Investigations by Hatch and coworkers (*13,14*) and by Glasziou (*9*) have clarified roles of invertase in sugar movement and accumulation.

Recent *in vitro* studies have shown that the invertase and amylase systems of cane are both plentiful and rugged (*4,5*). However, these enzymes were readily inhibited by mercury and iodide, and they were stimulated by manganese.[3] It was thus considered possible that the same materials might cause enzyme suppression or stimulation if supplied to living cane. To test this hypothesis, evaluations were made of invertase and amylase from cane which had received Mn and Hg as foliar sprays and nutrient-solution supplements.

MATERIALS AND METHODS

Plants of the variety M.336 were grown by sand culture in the greenhouse, according to procedures established earlier (*6*). Two experiments were conducted; one, involving foliar applications of Mn and Hg, and a second with supplemental additions of Mn and Hg to the standard nutrient solutions.[4]

Twelve-week-old plants were employed for experiment 1. Manganese

[1] Associate Plant Physiologist, Agricultural Experiment Station, University of Puerto Rico, Rio Piedras, P.R.

[2] Italic numbers in parentheses refer to Literature Cited pp. 34–5.

[3] Abbreviations: Mercury, (Hg); iodide, (I); manganese, (Mn); glucose-1-phosphate, (G-1-P); adenosine triphosphate, (ATP); β-glycerophosphate, (β-GP); and parts per million, (p.p.m.).

[4] All plants received the following macronutrients, 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: Boron, 0.05; copper, 0.02; manganese, 0.50; zinc, 0.05; molybdenum, 0.01; and iron, 0.50.

and Hg were applied in distilled water at rates of 50 and 1,000 p.p.m., using "tween 20" as wetting agent at the rate of 0.10 ml. per 100 ml. of solution. Control plants received distilled water plus wetting agent. Each solution was applied with a hand sprayer until all above-sand portions of the plants were thoroughly wet and run-off had begun.

Nutrient solutions for the second experiment were provided on Tuesdays, Thursdays, and Saturdays, beginning with 10-week-old plants and continuing over a period of 5 weeks. Distilled water was given on alternate days. Manganese levels were 2.5 and 25.0 p.p.m., whereas Hg variables were established at 0.05 and 1.0 p.p.m. Arsenate, W, and I were also tested as nutrient-solution supplements, each at concentrations of 0.05 and 1.0 p.p.m. All treatments were replicated 3 times, and the experimental design was a completely randomized block for each study.

Leaf and meristem tissues were harvested during both experiments for sugar and enzyme analyses. Sampling technique and subsequent freeze-drying procedures have been described previously (*6*). Samples were taken from experiment 1 a few moments prior to foliar treatments, and again 3, 9, and 27 days thereafter. Experiment 2 was harvested once after the plants had been treated for 5 weeks.

Sugars and enzyme protein were extracted from powdered, freeze-dried tissues as described in an earlier report (*1*). Total ketoses were determined with clarified extracts by the resorcinol method of Roe (*16*), and sucrose by the modification of Cardini *et al.* (*8*). Fructose was estimated by subtracting sucrose values from those of total ketose. Protein was precipitated with ammonium sulfate from clarified extracts, at pH 7.0 and 22° C., and taken up in distilled water. The technique of Sutherland *et al.* (*17*) was used to measure protein content.

Enzymes assayed during the present investigations included amylase, invertase, and the acid phosphatases which hydrolyze β-GP, ATP, and G-1-P. Phosphatase procedures were described previously (*1*), as were those for amylase (*5*) and invertase (*4*).

All data gathered during these studies were subjected to statistical analysis of variance, and means were compared by the Student-Newman-Keuls Q test (table 1).

RESULTS AND DISCUSSION

EXPERIMENT 1: FOLIAR APPLICATION OF MANGANESE AND MERCURY

Leaf and meristem sugars

Whereas manganese had no effect on leaf and meristem sugar levels, high Hg caused a pronounced deterioration of leaf-sucrose content (table 2, item 10). This was still evident 27 days after treatment (fig. 1). The

TABLE 1.—*Summary of significant mean differences among sugar, enzyme, and protein values for sugarcane treated with regulators of invertase and amylase*[1]

Data classification	Experiment 1 (foliar Mn and Hg) results on days following treatment indicated			
	0	3	9	27
Leaf sugars				
Total ketose		1 > 5* 4 > 5*		4 > 5* 3 > 5* 2 > 5* 1 > 5*
Sucrose		1 > 5* 4 > 5*		2 > 5** 4 > 5** 3 > 5** 1 > 5**
Fructose				5 > 4** 5 > 3** 5 > 1** 5 > 2**
Meristem sugars				
Total ketose				2 > 1*
Sucrose			3 > 5** 2 > 5** 3 > 1* 2 > 1*	
Leaf enzymes				
ATP-ase		2 > 1* 2 > 5*		
G-1-P ase	1 > 2** 1 > 5** 1 > 4** 1 > 3**		1 > 4** 1 > 3* 1 > 2*	
Amylase		2 > 4** 2 > 5** 3 > 5** 1 > 5** 4 > 5** 3 > 4*	3 > 5** 1 > 5** 2 > 5* 4 > 5*	1 > 2* 1 > 5* 1 > 3* 1 > 4*

Meristem enzymes				
ATP-ase				5 > 4*
				5 > 1*
				5 > 3*
G-1-P ase	5 > 3**	5 > 3**	1 > 3*	5 > 4**
	5 > 1*	5 > 4**	1 > 2*	5 > 3**
	5 > 2*	1 > 3**	1 > 4*	2 > 4**
	5 > 4*	1 > 4**		2 > 3**
		5 > 2*		1 > 4*
		1 > 2*		1 > 3*
		2 > 3*		
		2 > 4*		
Amylase		2 > 1**	5 > 4**	
		2 > 3**	5 > 3*	
		2 > 4**	5 > 2*	
		2 > 5**	1 > 3*	
			1 > 2*	
			1 > 4*	
Invertase	2 > 3*	2 > 5**	1 > 5**	2 > 4*
	5 > 3*	3 > 5**	1 > 3**	2 > 5*
		1 > 5**	1 > 2**	2 > 3*
		4 > 5**	1 > 4**	2 > 1*
		2 > 4*	5 > 4*	
Leaf protein		5 > 1**		
		5 > 4**		
		5 > 3**		
		5 > 2**		
Meristem protein			4 > 5*	3 > 5*
			4 > 1*	3 > 2*
				1 > 5*
				1 > 2*
				4 > 5*
				4 > 2*

TABLE 1.—*Continued*

Tissue	Experiment 2 (nutrient-solution supplements) significant mean differences are summarized for the indicated criteria—							
	Total ketose	Sucrose	Fructose	ATP-ase	G-1-P ase	Amylase	Invertase	Protein
Leaf				8 > 7* 6 > 7* 4 > 7* 11 > 7*	1 > 4** 1 > 6** 1 > 7** 1 > 3** 1 > 11** 1 > 8** 1 > 9** 1 > 10** 2 > 5**, etc. 5 > 10* 5 > 9* 4 > 10* 6 > 10* 7 > 10*	11 > 5** 9 > 5** 8 > 5** 4 > 5** 2 > 5** 7 > 5** 1 > 5* 10 > 5* 3 > 5* 6 > 5* 5 > 5*		6 > 8** 5 > 8** 6 > 11* 5 > 11* 4 > 8*
Meristem	7 > 8** 2 > 8** 7 > 9* 2 > 9* 1 > 9*	2 > 8** 7 > 8** 2 > 9* 2 > 11* 2 > 10* 7 > 9* 7 > 11* 7 > 10* 1 > 10* 1 > 8*	6 > 9*	1 > 5*	7 > 8** 7 > 2** 7 > 1** 7 > 3** 7 > 4** 7 > 5** 7 > 6** 11 > 3**, etc. 9 > 3**, etc. 10 > 3**, etc. 8 > 5** 8 > 6** 2 > 5** 2 > 6** 1 > 6** 7 > 11*, etc. 2 > 3*, etc. 1 > 3*, etc.	7 > 10** 7 > 11** 7 > 8** 7 > 6** 7 > 3** 7 > 4** 7 > 5** 9 > 6**, etc. 1 > 6**, etc. 2 > 6**, etc. 9 > 8*, etc. 1 > 8*, etc. 2 > 6*, etc. 10 > 5*	10 > 7** 10 > 3** 10 > 4** 10 > 2** 10 > 6** 10 > 5** 10 > 1** 8 > 2**, etc. 9 > 6**, etc. 11 > 5** 11 > 1** 9 > 3*, etc. 11 > 4*, etc.	5 > 7** 6 > 7* 3 > 7* 8 > 7* 4 > 7* 11 > 7*

[1] The numbers and symbols used to prepare this table are defined as follows: For experiment 1, numbers 1 through 5 represent mean values for control, Mn, and Hg treatments. Experiment 1, No. 1 = Control; 2 = Mn, 50 p.p.m.; 3 = Mn, 1,000 p.p.m.; 4 = Hg, 50, p.p.m.; 5 = Hg, 1,000 p.p.m. For experiment 2, numbers 1 through 11 refer to mean values for control, I, W, Mn, As, and Hg treatments. No. 1 = control; 2 = I, 0.05 p.p.m.; 3 = I, 1.0 p.p.m.; 4 = W, 0.05 p.p.m.; 5 = W, 1.0 p.p.m.; 6 = Mn, 2.5 p.p.m.; 7 = Mn, 25.0 p.p.m.; 8 = As, 0.05 p.p.m.; 9 = As, 1.0 p.p.m.; 10 = Hg, 0.05 p.p.m.; 11 = Hg, 1.0 p.p.m. The symbol > appearing between 2 figures indicates that the first mean was of greater magnitude than the second. Significant mean difference at the 5-percent level is denoted by 1 asterisk, and at the 1-percent level by 2 asterisks.

loss of sucrose was not at once accompanied by increased fructose, as one might have expected if a simple inversion were involved. Curiously, a marked rise in fructose did appear at 27 days (table 2, item 15), at the same period when control plants experienced their only fructose decline (table 2, item 11). Mercury thus transmuted the control response for fructose, while maintaining a continued suppression of sucrose.

Meristem sugar data (table 3) exemplify the difficulty of diverting immature cane from a physiological course it has set itself upon. The only

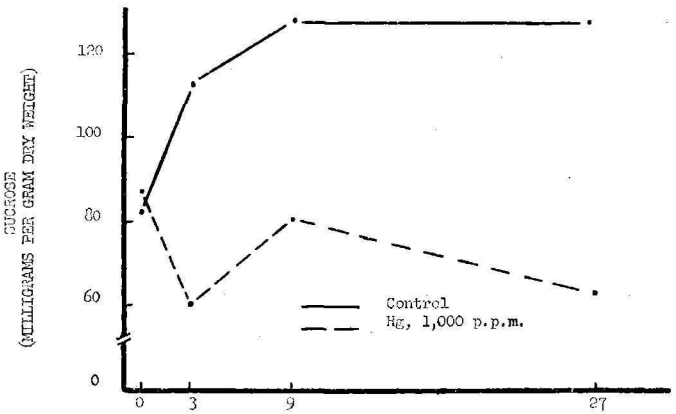

FIG. 1.—Decline of leaf-sucrose content of immature sugarcane following foliar application of mercury.

consistent trends among meristem sugars were an abrupt increase in sucrose at 27 days, coupled with a fructose decline during the same period (table 3, items 6 to 10 *vs.* items 12 to 15). High Mn lessened the sucrose changes and Hg increased them (fig. 2). But the overall trend, as established by control plants, remained essentially the same. This was particularly surprising with respect to the plants receiving high Hg, for their foliage at once appeared so scorched and desiccated that it was doubtful whether they would live for the duration of the study. Meristem-sugar data give no inkling that any severe shock had occurred. This again suggests that the plants had been able to confine Hg effects to their leaves.

Leaf and meristem enzymes

Neither ATP-ase nor G-1-P ase was consistently affected by the Mn and Hg treatments in leaf and meristem. Amylase was markedly suppressed by both levels of Hg in leaves (table 4, items 14 and 15; figure 3). As was true of sucrose decline in leaves, the leaf-amylase response was not repeated in meristem. However, some influence was being exerted upon meristem

TABLE 2.—*Leaf sugar content of immature sugarcane following foliar application of manganese and mercury*[1]

Item No.	Sugar	Treatment[2]	Results on days following treatment indicated				Mean
			0	3	9	27	
1	Total ketose	Control	127.4	151.5	169.9	150.3	149.8
2		Mn 50	117.9	127.1	168.6	154.0	141.9
3		Mn 1,000	121.9	120.3	176.3	159.0	144.4
4		Hg 50	126.8	137.7	171.7	165.8	150.5
5		Hg 1,000	131.8	104.1	122.6	129.2	121.9
6	Sucrose	Control	82.7	113.3	127.9	127.5	112.9
7		Mn 50	75.8	90.2	133.4	132.8	108.1
8		Mn 1,000	81.5	87.3	107.1	130.0	101.5
9		Hg 50	78.2	96.8	124.8	131.5	107.8
10		Hg 1,000	87.6	60.1	80.4	62.4	72.6
11	Fructose	Control	44.7	38.2	40.7	22.8	36.6
12		Mn 50	41.9	27.2	35.2	21.1	33.9
13		Mn 1,000	40.5	33.0	35.9	29.0	31.6
14		Hg 50	48.7	40.9	46.9	34.3	42.7
15		Hg 1,000	44.2	44.1	42.3	66.9	49.4

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

[2] The numerals 50 and 1,000 signify concentrations of 50 p.p.m. and 1,000 p.p.m., respectively.

amylase by Hg, as evidenced by an accentuation of enzyme fluctuations with low Hg, and by a moderating effect upon amylase from high Hg (table 5, items 14 and 15).

Meristem invertase was not inhibited by Hg nor stimulated by Mn. Rather, Hg and Mn sufficed only to restrain treated plants from experiencing invertase extremes such as that shown by control plants at 9 days (table 5). Such qualifying effects upon amylase and invertase by enzyme regulators are a far cry from the vivid inhibitory and stimulating effects observed *in vitro*. Notwithstanding, it would hardly be prudent to discount

the theory that Mn and Hg can influence enzymes in living tissues, nor to ponder what advantages such *in vivo*[5] action might one day offer the wise investigator.

TABLE 3.—*Meristem sugar content of immature sugarcane following foliar application of manganese and mercury*[1]

Item No.	Sugar	Treatment[2]	Results on days following treatment indicated				Mean
			0	3	9	27	
1	Total ketose	Control	326.7	304.2	343.9	269.9	311.2
2		Mn 50	271.4	316.8	316.8	329.8	333.7
3		Mn 1,000	390.0	354.5	328.3	284.3	339.5
4		Hg 50	370.5	324.6	321.9	303.8	330.2
5		Hg 1,000	384.4	336.7	281.8	308.9	327.9
6	Sucrose	Control	84.1	79.3	83.2	123.0	91.1
7		Mn 50	94.0	91.4	106.2	153.0	111.2
8		Mn 1,000	91.9	104.9	107.2	124.3	107.3
9		Hg 50	87.5	72.8	88.8	139.5	97.2
10		Hg 1,000	82.8	84.9	72.9	147.7	97.1
11	Fructose	Control	242.7	224.9	260.9	146.9	218.9
12		Mn 50	277.3	225.3	210.6	176.4	222.4
13		Mn 1,000	286.0	262.6	221.0	159.5	232.0
14		Hg 50	282.9	251.8	233.1	164.5	233.1
15		Hg 1,000	301.6	251.8	208.9	161.2	233.4

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

[2] The numerals 50 and 1,000 signify concentrations of 50 p.p.m. and 1,000 p.p.m., respectively.

EXPERIMENT 2: NUTRIENT-SOLUTION SUPPLEMENTS

Leaf and meristem sugars

Meristem tissues rather than leaves revealed the greatest sugar changes when Hg and Mn were provided as nutrient supplements. Low Hg caused a slight sucrose decline in leaves (table 6, item 4), whereas meristem sucrose was severely lowered by both Hg treatments (table 6, items 4 and 5). Fructose did not increase, so again inversion does not appear to have been involved. Low Mn likewise induced a sucrose decline in meristem (table 6,

[5] Technically, no true *in vivo* enzyme measurements were taken during this study. The term *in vitro*, as herein employed, refers to the effects of enzyme regulators upon catalysts in the test tube, and *in vivo* refers to the properties of enzymes extracted from plants which had been supplied with enzyme regulators while still alive.

item 2), and this is more nearly accounted for by a concurrent increase of fructose.

However, the most severe change in meristem sugar content was caused by low As, a treatment which lowered sucrose content to less than half of control values (table 6, item 10). Arsenate does not affect cane invertase *in vitro* (*13*), and the failure of low As to increase fructose again tends to discount invertase as the mechanism of sucrose decline.

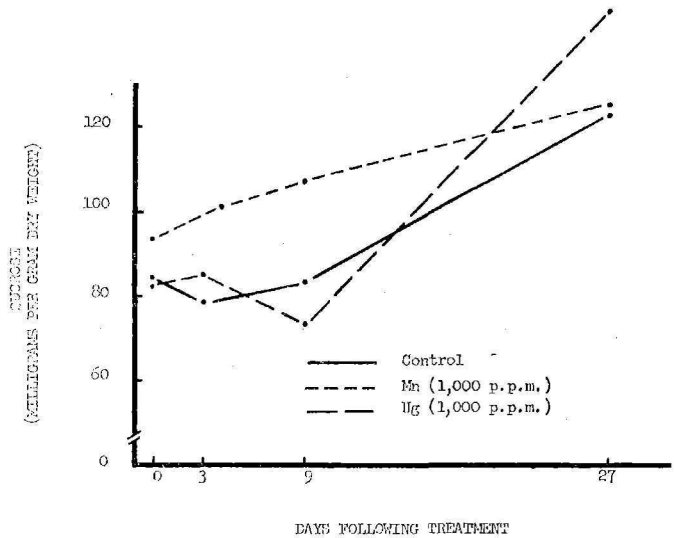

FIG. 2.—Sucrose changes in meristem of immature sugarcane following foliar application of manganese.

The only treatment at all inclined toward increasing meristem sucrose was the low W supplement (table 6, item 8). This increase was apparently mediated at the expense of fructose content. Conversely, the very slight sucrose increases caused by low I and high Mn (table 6, items 6 and 3) were the result of a general increase of total ketose.

Leaf and meristem enzymes

Manganese and mercury supplied through the roots failed to influence amylase and invertase in a manner anticipated from *in vitro* studies. Mercury, the amylase-invertase inhibitor, greatly stimulated meristem invertase (table 7, item 5), whereas neither of the Mn treatments apprecia-

bly affected amylase from leaf or meristem. High Mn managed to stimulate invertase in meristem, true to hypothesis, but not nearly so well as did the inhibitor Hg. In fact, the very appearance of a nutrient-solution additive usually served to activate invertase. However, such results need be deemed negative only to the extent that we do not know how to interpret them.

TABLE 4.—*Mean specific-activity values for leaf enzymes of immature sugarcane following foliar application of manganese and mercury*[1]

Item No.	Enzyme	Treatment[2]	Results on days following treatment indicated				Mean
			0	3	9	27	
1	ATP-ase	Control	28.9	29.7	28.4	30.1	29.3
2		Mn 50	27.4	41.4	24.9	24.4	29.5
3		Mn 1,000	25.4	32.6	27.8	24.2	27.5
4		Hg 50	27.3	32.4	26.2	23.5	27.4
5		Hg 1,000	23.9	28.4	28.9	33.8	28.8
6	G-1-P ase	Control	6.5	6.0	6.7	6.3	6.4
7		Mn 50	5.0	6.1	5.2	5.2	5.4
8		Mn 1,000	4.7	5.1	5.3	4.3	4.8
9		Hg 50	4.5	5.5	4.5	4.5	4.7
10		Hg 1,000	4.8	6.1	6.3	5.6	5.7
11	Amylase	Control	95.1	103.3	146.7	189.0	133.5
12		Mn 50	86.6	131.0	112.3	150.6	120.1
13		Mn 1,000	81.1	116.2	157.6	137.3	123.1
14		Hg 50	88.7	81.5	94.9	134.6	99.9
15		Hg 1,000	85.7	35.7	32.9	141.3	73.9

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

[2] The numerals 50 and 1,000 signify concentrations of 50 p.p.m. and 1,000 p.p.m., respectively.

Protein analyses, experiments 1 and 2

Reasons for the failure of Mn and Hg to yield predictable effects upon invertase and amylase cannot be confirmed on the basis of cell-free experiments. True, high Hg did suppress amylase in leaves, but only in concentrations so high as to threaten the life of the plant, and thus did not really support *in vitro* studies which suggested almost complete inhibition by mere traces of additive. Again, the expected comment here is that these *in vivo* experiments are only preliminary, that more Hg and Mn sources must be tried, at different concentrations and with more mature plants. This is not so. If *in vitro* results were at all applicable to living tissues, ample opportunity was given for verification.

Unexpected *in vivo* effects of enzyme regulators have been encountered earlier with immature cane (*7,6*). It was suggested that both the synthesis of new enzyme protein and the *in vivo* retention of biochemical enzyme sequences, in contrast to isolation imposed by the test tube, accounted for discrepancies between test-tube based hypotheses and *in vivo* findings. Gross protein data from the present studies strengthens our belief that treatment additives had influenced the production of enzyme protein.

It will be recalled that the only treatment of consequence during experi-

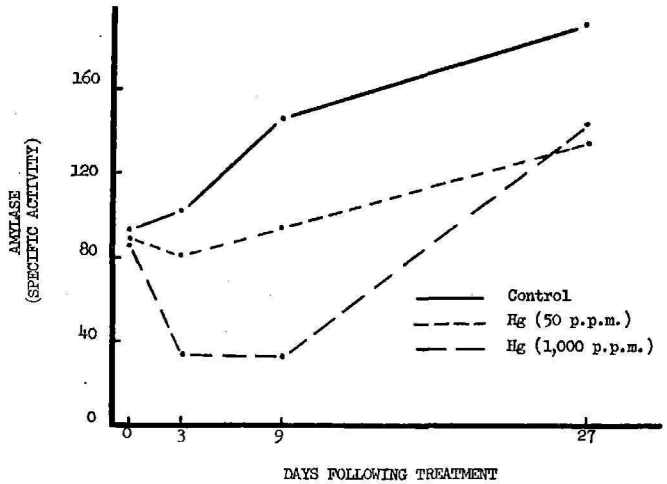

FIG. 3.—Inhibition of amylase from leaves of immature sugarcane following foliar application of mercury.

ment I was the foliar application of high Hg, with subsequent deterioration of sucrose content and severe inhibition of amylase. This treatment likewise induced a relatively large increase of protein (table 8, item 5). In meristem, where less drastic sugar and enzyme changes had been recorded, there were a general increase of protein among all plants receiving foliar additives (table 8, items 7–10).[6]

With the one exception of high Mn, plants receiving additives as nutrient-solution supplements also revealed a general increase of meristem

[6] The protein increases ranged from 18 to 43 percent and were not in themselves large. However, it should be remembered that these are gross protein figures, including all enzyme protein as well as that which is noncatalytic. Within these ranges the content of any single enzyme could vary manyfold.

protein (table 9). The contention that additives might stimulate protein synthesis is well enough vindicated. Supposedly, the initial appearance of an enzyme inhibitor can stimulate a recuperative mechanism which more than compensates for enzyme inactivated. Yet, for all we know of cane enzymes, which is not much, the materials supplied as "inhibitors" might ac-

TABLE 5.—*Mean specific-activity values for meristem enzymes of immature sugarcane following foliar application of manganese and mercury*[1]

Item No.	Enzyme	Treatment[2]	Results on days following treatment indicated				Mean
			0	3	9	27	
1	ATP-ase	Control	25.1	34.7	25.9	23.0	27.2
2		Mn 50	27.3	45.5	20.7	29.9	30.9
3		Mn 1,000	24.1	31.1	21.2	22.9	24.8
4		Hg 50	23.7	34.2	17.4	23.1	24.6
5		Hg 1,000	38.0	29.8	22.5	32.3	30.7
6	G-1-P ase	Control	8.5	6.7	7.7	7.4	7.6
7		Mn 50	6.7	5.5	4.6	8.2	6.3
8		Mn 1,000	4.8	4.3	4.8	5.6	4.9
9		Hg 50	6.1	3.6	3.9	6.0	4.9
10		Hg 1,000	15.8	6.9	5.9	9.0	9.4
11	Amylase	Control	56.5	89.5	65.0	68.0	68.5
12		Mn 50	58.8	111.2	48.2	77.4	73.9
13		Mn 1,000	51.5	85.3	51.9	67.6	64.1
14		Hg 50	54.3	84.6	42.9	67.9	62.4
15		Hg 1,000	56.1	75.9	71.4	76.0	69.9
16	Invertase	Control	20.1	11.9	27.2	8.5	16.7
17		Mn 50	24.2	13.8	14.5	12.7	16.3
18		Mn 1,000	17.0	12.0	15.9	8.9	13.5
19		Hg 50	19.7	11.1	13.9	9.6	13.6
20		Hg 1,000	23.7	8.6	17.7	9.2	14.8

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

[2] The numerals 50 and 1,000 signify concentrations of 50 p.p.m. and 1,000 p.p.m., respectively.

tually be true activators of a catalyst busy in the sequence of protein-forming reactions. A third consideration is the increased synthesis of endogenous enzyme inhibitors caused by treatments herein employed. Such inhibitors themselves need not be catalytic, nor even protein, yet as products of reactions independent from those of protein formation, they would help account for the *in vivo* vs. *in vitro* discrepancies recorded here.

Signs of a nonprotein entity are evident in the data already gathered.

TABLE 6.—*Sugar content of immature sugarcane supplied with variable iodide, tungsten, manganese, arsenate, and mercury in sand culture*[1]

Item No.	Treatment[2]	Leaf sugars			Meristem sugars		
		Total ketose	Sucrose	Fructose	Total ketose	Sucrose	Fructose
1	Control	104.6	83.9	20.7	299.9	111.4	188.5
2	Mn_1	101.3	82.5	18.8	273.0	74.1	198.9
3	Mn_2	114.0	85.9	28.1	312.9	120.9	191.9
4	Hg_1	100.3	71.1	29.2	233.8	53.3	180.5
5	Hg_2	99.3	77.5	21.7	240.9	61.1	179.8
6	I_1	98.6	80.8	17.8	312.8	123.5	189.3
7	I_2	105.7	78.1	27.6	279.9	88.4	191.5
8	W_1	104.1	82.8	21.3	272.6	130.0	142.6
9	W_2	111.3	86.3	25.0	263.5	94.0	169.4
10	As_1	97.8	78.6	20.4	193.3	45.2	137.8
11	As_2	102.1	74.5	28.8	201.5	62.8	138.7

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

[2] The numerals 1 and 2 refer to low and high additive levels. In each instance this is 0.05 and 1.0 p.p.m., respectively, with the exception of manganese, which was supplied at 2.5 and 25.0 p.p.m.

TABLE 7.—*Mean specific-activity values for leaf and meristem enzymes of immature sugarcane supplied with variable iodide, tungsten, manganese, arsenate, and mercury in sand culture*[1]

Item No.	Treatment[2]	Leaf enzymes			Meristem enzymes			
		ATP-ase	G-1-P-ase	Amylase	ATP-ase	G-1-Pase	Amylase	Invertase
1	Control	47.9	11.6	207.7	27.6	6.8	135.9	12.3
2	Mn_1	54.9	9.0	195.6	20.2	4.7	100.7	14.2
3	Mn_2	38.3	8.9	218.9	24.8	9.4	154.0	17.5
4	Hg_1	45.9	6.6	204.9	24.1	7.7	119.6	28.8
5	Hg_2	52.3	8.4	254.9	22.2	8.0	116.4	23.2
6	I_1	49.3	11.5	222.8	25.7	7.0	132.9	15.0
7	I_2	51.0	8.6	199.4	20.7	5.6	100.2	17.2
8	W_1	54.3	9.0	232.1	21.6	5.6	96.4	16.5
9	W_2	47.6	9.6	145.7	18.2	4.9	93.8	12.9
10	As_1	57.5	7.9	243.1	21.9	7.1	111.5	25.0
11	As_2	48.1	7.1	243.2	25.4	7.9	137.6	23.7

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

[2] The numerals 1 and 2 refer to low and high additive levels. In each instance, this is 0.05 and 1.0 p.p.m., respectively, with the exception of manganese, which was supplied at 2.5 and 25.0 p.p.m.

TABLE 8.—*Leaf and meristem protein content of immature sugarcane following foliar application of manganese and mercury*[1]

Item No.	Data classification—	Treatment[2]	Results on days following treatment indicated				Mean
			0	3	9	27	
1	Leaf protein	Control	5.78	5.66	5.09	5.43	5.49
2		Mn 50	6.40	4.66	5.95	6.07	5.77
3		Mn 1,000	6.56	5.00	4.99	6.81	5.84
4		Hg 50	5.99	5.38	5.99	6.35	5.93
5		Hg 1,000	6.49	6.93	6.74	7.43	6.89
6	Meristem protein	Control	18.64	14.80	15.76	14.92	16.03
7		Mn 50	17.92	12.40	26.68	19.12	19.03
8		Mn 1,000	21.64	16.00	26.84	26.20	22.67
9		Hg 50	19.96	18.60	29.12	24.12	22.95
10		Hg 1,000	20.40	22.40	16.60	19.40	19.70

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

[2] The numerals 50 and 1,000 signify concentrations of 50 p.p.m. and 1,000 p.p.m., respectively.

TABLE 9.—*Leaf and meristem protein content of immature sugarcane supplied with variable iodide, tungsten, manganese, arsenate, and mercury in sand culture*[1]

Data classification	Control	Results with variable additives, supplied as indicated in p.p.m.—[2]										Mean for variable additives
		Mn_1	Mn_2	Hg_1	Hg_2	I_1	I_2	W_1	W_2	As_1	As_2	
Leaf protein	3.21	3.75	3.22	3.16	2.72	3.22	3.20	3.13	3.70	2.40	3.29	3.21
Meristem protein	16.72	21.32	14.00	18.00	19.48	17.04	20.92	19.96	22.44	20.40	18.56	19.21

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

[2] The numerals 1 and 2 refer to low- and high-additive levels. In each instance this is 0.05 and 1.0 p.p.m., respectively, with the exception of manganese, which was supplied at 2.5 and 25.0 p.p.m.

For example, the high Hg treatment which retarded leaf sucrose and amylase in experiment 1 caused a major protein increase some time after the 9-day harvest, whereas both the sucrose and amylase effects were well established at 3 days. Amylase activity was again on the rise when the major protein increase was recorded at 27 days (fig. 3). This suggests that

increased protein formation here was a corrective measure, a consequence of the severe Hg effect rather than a means of its execution. Again, an investigator might have concluded that the stimulation of invertase during experiment 2 by As and Hg (table 7, items 4 and 5, 10 and 11) was related to increased protein as a response to these same additives (table 9). Yet invertase activity was relatively low among plants receiving high W and low Mn (table 7, items 9 and 2), and these treatments induced the highest protein increases of that experiment (table 9).

Gross protein measurements cannot distinguish for us the comparative effects of Hg, As, W, and Mn upon individual components of the increased protein, and this is the key for advancing such work in the future. Within a group of newly formed proteins, any of these treatments might have caused an increase of invertase, and any other treatment an increase of invertase inhibitor. The next logical step will be the study of enzyme protein formation under controlled conditions, where the individual effects of additives upon the synthesis of general protein and specific catalysts can be measured.

SUMMARY

Immature sugarcane grown in sand culture was treated with manganese, an *in vitro* activator of amylase and invertase, and with mercury, an *in vitro* inhibitor of the same enzymes. Both elements were supplied as foliar sprays and as nutrient-solution supplements. The objective was to determine whether *in vivo* effects would be equivalent to those observed in the test tube, and to determine the effects of these elements on sugar content. Leaf and meristem tissues were frozen and lyophilized for sugar and enzyme assay.

Mercury solution of 1,000-p.p.m. concentration caused a marked suppression of leaf amylase activity and sucrose content which persisted 27 days after treatment. The major mercury effect was confined to the leaves. In meristem tissues, sucrose changes as established by control plants were enhanced by mercury and alleviated by manganese. Mercury at 1,000 p.p.m. caused a moderate invertase suppression which disappeared by 27 days after treatment.

Plants receiving mercury and manganese as nutrient-solution supplements experienced major sugar changes in meristem rather than leaves. Low manganese, and both high and low mercury, greatly suppressed sucrose content. Arsenate, which was also being tested, likewise caused marked deterioration of meristem sucrose. Invertase was generally stimulated among plants receiving additives through their roots. Amylase was not affected. Mercury, an extremely efficient invertase inhibitor in the test tube, more than doubled invertase activity in plants receiving 0.05 p.p.m. of mercury in their nutrient solutions.

In vivo effects of manganese and mercury did not agree well with expectations based on *in vitro* observations. Even in those instances where the anticipated results were gained, these required far greater concentrations of additive than should have been necessary. Nevertheless, definite influences were exerted upon both amylase and invertase.

Discrepancies between *in vivo* and *in vitro* results are discussed. It is suggested that these differences are attributable to the positioning of *in vivo* catalysts in reaction sequences, to the stimulation of protein production by treatment additives, and to the increased production of non-protein, endogenous enzyme activators and inhibitors.

RESUMEN

Se trató caña inmadura sembrada en arena, con manganeso (agente activante de la amilasa y la invertasa *in vitro*) y con mercurio (agente inhibidor de las mismas enzimas *in vitro*). Ambos elementos se aplicaron en forma de aspersión foliar y de solución nutritiva suplementaria. El propósito fue determinar si los efectos *in vivo* serían los mismos que se obtuvieron en el laboratorio, y determinar también el efecto de estos elementos sobre el contenido de azúcar. Se congelaron y liofilizaron tejidos foliares y del meristemo para el análisis del azúcar y las enzimas.

Una solución de mercurio a una concentración de 1,000 p.p.m. redujo marcadamente la actividad de la amilasa y de la sacarosa en la hoja, lo cual persistió hasta 27 días después del tratamiento. El efecto principal del mercurio se limitó a las hojas. En los tejidos del meristemo, el mercurio aceleró y el manganeso redujo el patrón de cambios en la sacarosa, según lo establecieron las plantas-testigo. El mercurio, a razón de 1,000 p.p.m., redujo moderadamente la acción de la invertasa, efecto que desapareció 27 días después del tratamiento.

Las plantas que recibieron mercurio y manganeso en forma de solución nutritiva suplementaria sufrieron los mayores cambios de azúcar en el meristemo mas bien que en las hojas. Un bajo nivel de manganeso y niveles altos y bajos de mercurio, limitaron considerablemente el contenido de sacarosa. El arseniato, que también se puso a prueba, causó igualmente un marcado deterioro de la sacarosa en el meristemo. En general, se estimuló la invertasa en aquellas plantas que recibieron aditivos por las raíces, sin que la amilasa se afectara. El mercurio, que es un inhibidor de la invertasa extraordinariamente eficaz en el laboratorio, aumentó más de dos veces la actividad de la invertasa en las plantas tratadas con unas soluciones nutritivas conteniendo 0.05 p.p.m de mercurio.

Los efectos del manganeso y el mercurio *in vivo* no correspondieron a lo que se esperaba, según las observaciones hechas *in vitro*. Aún en aquellas ocasiones en que se obtuvieron los resultados que se esperaban, se

requirieron concentraciones del aditivo en exceso a lo que debió ser necesario. No obstante, hubo influencias definidas sobre la amilasa y la invertasa.

Se discuten las discrepancias entre los resultados obtenidos *in vitro* é *in vivo*. Se sugiere la posibilidad de que estas diferencias se deban a la posicion de agentes catalíticos *in vivo* en secuencias reactivas, a la producción de proteína por el estímulo de los aditivos que se aplicaron; y al aumento en la producción de materias no-proteicas, activadores enzimáticos endógenos y agentes inhibidores.

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