Physiological Studies of Enzymes Catalyzing the Synthesis and Hydrolysis of Sucrose, Starch, and Phosphorylated Hexose in Sugarcane

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INTRODUCTION

Previous experiments have shown that treatments causing significant changes in leaf-sucrose content of cane also bring about marked variations in the starch-hydrolyzing enzyme amylase (*1,2*).[2] At that time we were not prepared to measure directly the biosynthesis of starch or sucrose, but the hypothesis was nevertheless put forward that starch serves as a source of free glucose which could be converted to sucrose precursors. It has also been pointed out that excessive hydrolysis of starch might deplete ATP reserves needed for the phosphorylation of glucose prior to sucrose biosynthesis (*3*).

Although an active starch-forming system in cane would utilize the same glucose-1-phosphate reserves eligible for, and required by, the pathways of sucrose synthesis, little attention has been given to either the biosynthesis of starch or starch content in sugarcane. One exception is posed by the variety Natal Uba, whose tendencies toward excessive starch accumulation have created difficulties for cane processors (*15*). Both the filtration and crystallization rates of cane juices are retarded by starch (*5,8*), and enzyme hydrolysis has been studied as a means of removing excess starch from the juices in the factory (*6*). Hartt (*10*) reported that amylase was less active in the meristematic tissue of the Uba variety than in other varieties tested. Workers in Puerto Rico have not considered starch as a factor of importance in sugarcane.

Recent experiments at our laboratory have revealed the presence of a starch phosphorylase in dialyzed protein preparations from sugarcane leaves. The enzyme is presumably identical to that described by Hanes (*9*), in pea seed and potato tubers, which catalyzes both the synthesis and breakdown of starch by reactions involving starch, phosphoric acid, and the Cori ester:

$$\text{Starch} + H_3PO_4 \underset{\text{Phosphorylase}}{\overset{\text{Starch}}{\rightleftharpoons}} \text{Glucose-1-phosphate}$$

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[2] Italic numbers in parentheses refer to Literature Cited, p. 75.

We have been able to demonstrate, with crude leaf preparations, the formation of sucrose from glucose-1-phosphate and fructose (*4*), presumably by a sucrose phosphorylase enzyme similar to that described by Hassid and coworkers (*11*) in *Pseudomonas saccharophila:*

$$\text{Glucose-1-phosphate} + \text{Fructose} \underset{\text{Phosphorylase}}{\overset{\text{Sucrose}}{\rightleftharpoons}} \text{Sucrose} + H_3PO_4$$

Evidence was also encountered which suggested the formation of sucrose through the intermediary of UDPG (uridine diphosphate glucose) according to the reactions developed by Leloir and coworkers (*7,12,14*):

1, UTP (Uridine triphosphate) + Glucose-1-phosphate →

UDPG + Pyrophosphate

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

The obvious importance of glucose-1-phosphate in the formation of sucrose and starch led us to investigate further the reactions in which this compound is utilized in sugarcane. This paper summarizes our work with enzymes taking part in the synthesis of sucrose, starch, and glucose-6-phosphate, and additional enzymes which catalyze the hydrolysis of these and related products.

EXPERIMENTAL PROCEDURE

The water and nutritional status of 4- to 6-month-old sugarcane was purposely altered in order to induce changes in sucrose content, and, thereby, to permit us to study concurrently the enzymes associated with sucrose and starch transformations. Nutritional treatments involved lowering nitrate from 10.0 to 1.5 meq./liter, and potassium from 5.0 to 0 meq./liter. This technique contrasted with earlier experiments (*1*) in which low-nitrate and low-potassium levels were gradually approached over a period of 27 weeks. The decreasing water treatments were applied as follows: Each container received 1 liter of nutrient solution every morning at 8 a.m., and 2 liters of supplemental water at 2 p.m., until the plants were 4 months of age.

After harvesting the second set of pretreatment samples, the water supply was reduced to 1 liter of nutrient solution each day, and supplemental water every second day, for a period of 14 days. At this time the third set of samples was harvested. The water supply was then further reduced to 1 liter of nutrient solution daily, plus supplemental water every third day for another period of 14 days. The final two treatments consisted of supplemental water every fifth and seventh day, so that the final

set of samples was taken from plants which had received only one supplemental watering during the previous 14 days.

One-eye cuttings of the variety M.336 were planted in quartz sand on April 20, 1963, and all plants received a complete nutrient solution[3] until treatments were initiated on August 20, 1963. The sand was previously treated with 0.05 N HCl for 12 hours, and after thorough leaching with tapwater, it was placed in 5-gallon, polyethylene wastebaskets fitted with glass wool over the drainage outlets. Twelve PMA-treated cuttings were planted in each basket.[4] At 3 months the plants were thinned to the five or six most uniform canes of each container.

At 4 months the containers were divided into three groups, and half of the plants within each group were subjected to low nitrate, low potassium, and decreasing water, respectively. The remaining half of the plants within each group served as controls by continuing to receive the original nutrient and water supplies. Every group represented a completely randomized block. There were three replicates for each of the nutritional treatments and six replicates for the water treatments.

Initial leaf samples were taken 7 days before treatments were begun and subsequent samples were harvested at intervals of 7 or 14 days over a period of 63 days. Plants of the nutritional studies were sampled at 7-day intervals up to 28 days, and at 14-day intervals thereafter, while plants of the decreasing water study were sampled at 14-day intervals only. The leaf samples were frozen, lyophilized, and subsequently extracted by procedures described previously in detail (*1*). Leaf-protein preparations, enzyme assays, and sugar assays have also been thoroughly described in the previous paper (1).

Enzymes assayed during the present study included sucrose phosphorylase, starch phosphorylase, invertase, amylase, hexokinase, peroxidase, and the acid phosphatases which catalyze the hydrolysis of β-glycerophosphate, glucose-1-phosphate, and ATP. With the exception of starch phosphorylase, procedures for each of the assays have been described previously (*1*,*4*).

The starch-phosphorylase method used here was a modification of the technique of Lozano and Bonner (*13*), employing a standardized iodine-potassium iodide solution to detect colorimetrically the amount of starch

[3] Nutrient concentrations, expressed as milliequivalents per liter, were provided as follows: 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, 1.0.

[4] The cuttings were immersed in a 0.25-percent solution of PMA (phenyl mercury acetate), and allowed to remain for 10 minutes. The treatment is a general antiseptic against seedborne micro-organisms.

formed from glucose-1-phosphate. Each reaction mixture was composed of 0.5 ml. of succinate buffer (pH 6.5), 0.5 ml. of 0.1-molar glucose-1-phosphate, 0.1 ml. of 5-percent soluble starch,[5] and 0.5 ml. of enzyme preparation representing 0.1 to 0.2 mg. of protein. Control tubes received distilled water in place of enzyme.

The reaction was allowed to proceed for 20 minutes at 30° C., and was then terminated with 2 ml. of 10-percent trichloroacetic acid. The mixture was centrifuged at 3,000 r.p.m. for 10 minutes. From the supernatant liquid, 0.5 ml. was withdrawn and transferred to a second set of test tubes. Iodine solution was then added, and 10 minutes were allowed for color development. The solutions were diluted to 3 ml. and optical density was recorded at 580 mμ. The amount of starch formed was determined by reference to a standard curve representing 0.01 to 0.25 mg. of soluble starch. The starch phosphorylase-activity unit was defined as the amount of enzyme catalyzing the formation of 0.1 mg. of starch under the specified conditions of the assay. All enzyme determinations were converted to specific-activity values, units per milligram of protein.

The data collected from these experiments were subjected to statistical analysis of variance, and mean values were compared by the Student-Neuman-Keuls Q test.

RESULTS AND DISCUSSION

SUCROSE CONTENT *VS.* STARCH PHOSPHORYLASE

The low-water, -nitrate, and -potassium treatments induced only part of the desired sugar fluctuations, but nevertheless we were able to gain some insight into the roles being played by the enzymes under scrutiny. Mean values presented in table 1 indicate a slight but generally consistent sucrose increase in response to low nitrate and low potassium. Possibly enough nutrient reserves had accumulated during the 4 months of adequate supply so that the plants could withstand the relatively drastic treatments, at least for the duration of the study. Control values for sucrose fluctuated throughout the test period in a manner suggesting that the plants were not primarily concerned with sucrose production. Quite likely the hexose and hexose phosphate precursors of sucrose were still being drawn into other growth and development processes. However, certain sampling periods, such as the −7- and 35-day harvests for the low-nitrate study (figure 1), disclosed definite peaks and depressions for sucrose values, and these gave us an opportunity to evaluate the enzymes which were responsible.

[5] A peculiarity of this enzyme is its requirement of a small amount of starch to initiate, or "prime", a reaction of maximum activity.

TABLE 1.—*Sugar content of leaves from immature sugarcane supplied with variable nitrate and potassium in sand culture*[1]

Sugar[3]	Treatment	Results at days indicated following treatment[2]								Mean
		−7	0	7	14	21	35	49	63	
Total ketose	Control	118.8	103.8	96.6	77.7	93.9	74.7	99.6	93.5	94.6
	Low nitrate	121.3	101.9	99.4	88.0	95.7	93.3	98.8	89.1	98.5
	Control	118.1	102.5	101.1	93.8	92.1	79.4	102.2	95.6	98.1
	Low potassium	118.5	101.6	103.4	101.6	89.3	102.1	110.9	106.1	102.9
Sucrose	Control	103.5	92.3	59.2	52.7	73.4	48.0	78.1	76.7	72.9
	Low nitrate	113.5	86.6	70.4	61.1	79.4	75.7	91.1	80.6	82.3
	Control	108.2	87.9	63.9	60.2	83.7	49.9	78.7	84.8	77.2
	Low potassium	109.5	91.3	77.4	69.0	75.4	64.9	93.0	91.4	83.9
Fructose	Control	15.4	11.7	37.4	23.2	20.4	26.7	21.6	16.8	38.9
	Low nitrate	7.8	15.3	29.0	26.8	16.3	14.2	7.8	8.4	15.7
	Control	9.8	14.9	37.1	33.6	8.3	29.4	23.3	10.9	20.9
	Low potassium	9.0	10.3	25.9	32.7	14.2	37.1	17.5	14.7	20.2
Total reducing sugars	Control	38.7	26.8	44.4	27.9	28.7	31.1	28.7	21.7	31.0
	Low nitrate	34.7	24.2	45.8	32.5	29.8	32.2	22.5	19.7	30.2
	Control	26.4	26.0	43.6	34.6	28.0	34.8	36.8	22.9	31.6
	Low potassium	25.5	24.0	40.1	43.7	31.2	50.8	47.3	32.8	36.9
Glucose	Control	23.2	15.2	5.7	4.7	8.3	4.4	7.1	4.9	9.2
	Low nitrate	29.0	8.9	16.8	5.7	13.4	17.9	14.7	11.3	14.7
	Control	16.5	11.1	6.5	1.0	19.8	5.4	13.5	12.1	10.7
	Low potassium	16.5	13.6	14.2	11.0	16.9	13.7	29.8	28.0	18.0
Mean		58.2	48.4	50.9	44.1	45.4	51.1	54.7	45.6	

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

[2] Initial samples were taken 7 days prior to treatment and again a few moments before the treatments were begun. These 2 harvests are designated "−7" and "0", respectively, in the table.

[3] Each sugar is expressed as milligrams per gram dry weight.

A review of the grand mean values summarized in table 2 tells us that the slight sucrose increases from low nitrate and low potassium were accompanied by a general suppression of the acid phosphatases and of amylase. Both effects were observed previously and have been discussed in detail (*1*).

A relationship was encountered between leaf-sucrose content and starch-phosphorylase activity, but the relationship is not yet entirely clear. Possibly the primary reason for this was the inability of any treatment to affect the enzyme sucrose phosphorylase, the logical counterpart of any altered starch-phosphorylase reaction. The sugar and enzyme data compiled in tables 1 and 2 tell us that whenever an extremely high or low sucrose level was recorded, this was usually followed within 7 or 14 days by a

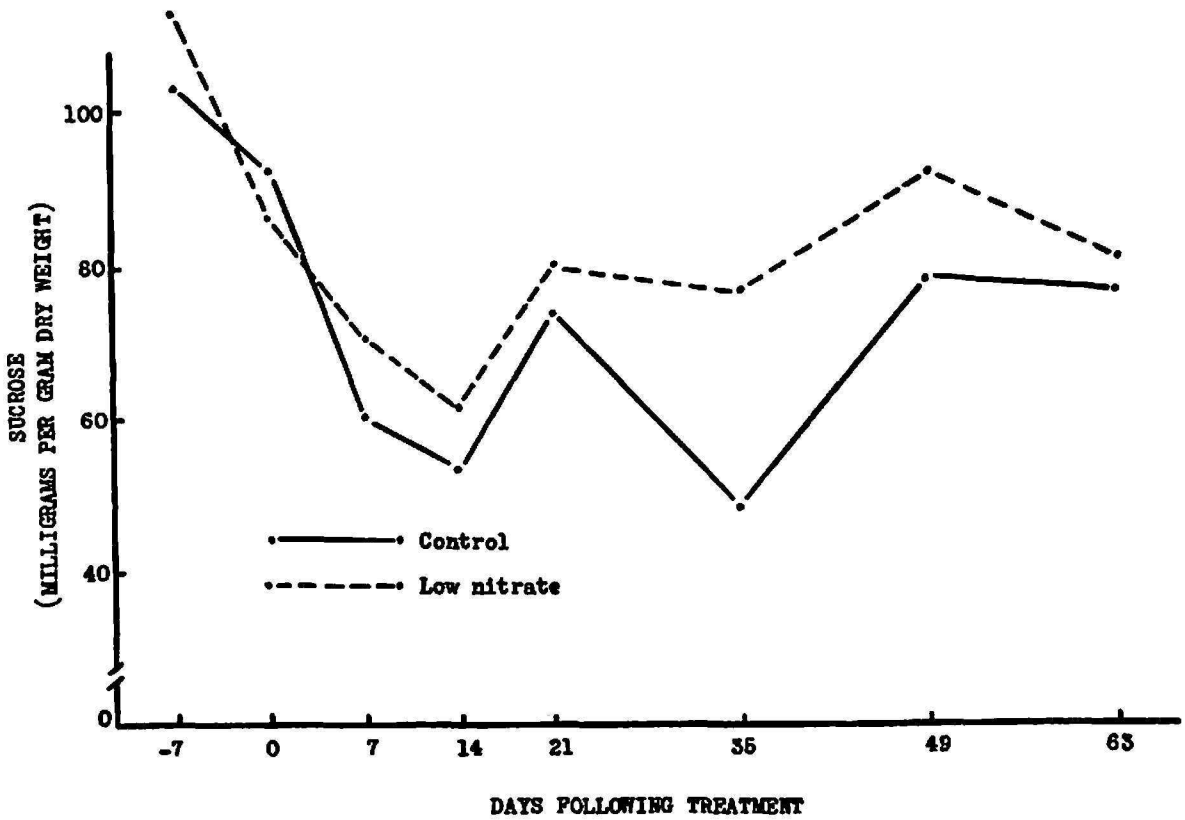


FIG. 1.—Leaf-sucrose content of 4-month-old sugar plants subjected to low nitrate (1.5 meq./liter) and control nitrate levels (10.0 meq./liter).

relatively extreme change in starch phosphorylase. The enzyme change was inversely related to the sucrose level. For example, the high-sucrose levels obtained for the nutritional experiments at −7 days were followed by reduced starch phosphorylase at the following harvest, whereas the low-sucrose values obtained at 35 days were followed by greatly stimulated starch phosphorylase. The same principle holds true for the control plants of the decreasing-water experiment (tables 3 and 4). Such evidence does not fully support a direct relationship between starch and sucrose synthesis, such as the following equations suggest:

$$\text{Starch} \rightleftharpoons \text{Glucose-1-phosphate} \overset{+\ \text{Fructose}}{\rightleftharpoons} \text{Sucrose}$$

TABLE 2.—*Mean specific-activity values for leaf enzymes of immature sugarcane supplied with variable nitrate and potassium in sand culture*[1]

Item No.	Enzyme	Treatment	Results at days indicated following treatment[2]								Mean
			−7	0	7	14	21	35	49	63	
1	β-glycerophosphatase	Control	10.9	25.3	38.1	30.0	25.8	31.5	50.9	46.1	32.3
		Low nitrate	8.6	25.6	35.2	31.1	17.2	21.6	23.8	27.3	23.8
		Control	9.1	33.6	44.9	37.9	22.1	34.4	49.3	54.9	35.8
		Low potassium	9.4	25.1	22.1	25.2	24.0	30.5	40.5	52.2	28.6
2	ATP-ase	Control	9.7	25.5	35.5	29.2	38.8	31.4	56.0	47.6	34.2
		Low nitrate	7.6	23.1	33.2	29.7	22.4	20.9	21.9	26.2	23.1
		Control	7.3	25.3	37.9	33.8	30.3	36.4	53.3	52.5	34.6
		Low potassium	6.7	21.7	17.7	20.3	29.5	25.1	39.1	53.2	26.9
3	Glucose-1-phosphatase	Control	4.7	5.8	10.2	8.2	9.4	8.3	12.7	12.2	8.9
		Low nitrate	3.8	6.5	8.7	8.0	7.6	7.1	6.5	9.6	7.2
		Control	3.8	4.4	6.1	5.7	6.5	7.0	10.4	14.1	7.3
		Low potassium	3.7	3.8	1.9	4.2	6.6	6.3	8.8	14.5	6.2
4	Hexokinase	Control	12.8	5.2	7.2	4.2	3.6	8.0	7.1	4.9	6.6
		Low nitrate	8.9	3.7	3.5	6.5	4.6	3.1	8.4	11.8	6.3
		Control	10.3	14.8	5.1	6.1	5.3	7.9	14.7	20.9	10.6
		Low potassium	14.7	13.4	4.1	9.9	7.2	10.2	20.4	32.7	14.1
5	Amylase	Control	40.4	40.4	119.8	63.5	66.1	68.5	116.5	64.4	72.5
		Low nitrate	33.0	41.4	72.2	63.5	56.5	42.8	38.8	43.9	49.0
		Control	29.1	41.1	98.4	100.2	51.0	70.4	106.6	93.4	73.8
		Low potassium	26.3	39.9	40.7	66.2	55.7	41.1	100.1	108.1	59.8

6	Starch phosphorylase	Control	6.0	4.4	7.2	12.1	11.9	6.4	20.3	10.0	9.8
		Low nitrate	9.2	8.8	7.6	7.1	10.2	8.2	9.7	13.8	9.3
		Control	5.1	2.7	3.7	5.5	7.2	6.3	14.1	5.3	6.2
		Low potassium	6.5	4.5	9.3	4.1	6.2	3.6	14.4	13.2	7.7
7	Sucrose phosphorylase	Control	.89	1.90	1.08	2.01	1.08	1.47	1.37	2.22	1.50
		Low nitrate	3.10	1.11	1.83	1.75	.44	5.02	.33	.38	1.75
		Control	1.21	1.18	1.81	.85	.80	.86	.37	.87	.99
		Low potassium	.86	1.62	.80	1.54	.57	.62	.97	.24	.90
8	Invertase	Control	1.5	1.5	2.0	1.4	2.0	1.5	1.3	1.9	1.6
		Low nitrate	1.4	1.2	1.4	1.5	1.6	1.2	.9	1.6	1.4
		Control	1.4	1.2	1.3	1.6	1.4	1.7	1.4	1.8	1.5
		Low potassium	1.7	1.0	1.9	1.4	1.2	1.4	1.4	1.9	1.5
9	Peroxidase	Control	4.7	10.7	13.2	7.4	42.3	6.2	5.6	4.2	11.8
		Low nitrate	4.2	6.8	12.4	12.3	40.5	3.7	3.5	4.8	11.0
		Control	3.8	8.7	11.9	11.1	47.3	6.3	7.1	4.3	12.6
		Low potassium	4.9	7.4	8.3	12.7	33.7	5.6	5.7	4.1	10.3
	Mean		8.8	13.6	20.2	18.5	19.4	16.1	24.3	23.9	

[1] Each figure represents the computed mean of 3 replicates. All enzymes are expressed in specific-activity values, with the exception of sucrose phosphorylase, and the latter is expressed as μmoles sucrose formed per milliliter of digest.

[2] Initial samples were harvested 7 days prior to treatment and again a few moments before the treatments were begun. These 2 harvests are designated "−7" and "0", respectively, in the table.

A direct relationship would have required that the stimulated or suppressed starch-phosphorylase reaction be in progress for some time before the altered sucrose level was detectable in the leaves. Our data thus far suggest that starch phosphorylase was retarded only after much of the available glucose-1-phosphate had already gone into sucrose, and was stimulated only after the sucrose-forming mechanism had slackened enough to allow additional glucose-1-phosphate to enter the starch-forming pathway.

TABLE 3.—*Leaf-sugar content of immature sugarcane subjected to variable water supply in sand culture*[1]

Sugar[2]	Treatment	Results at days indicated following initial treatment						Mean
		−14	0	14	28	42	56	
Total ketose	Control	117.4	98.9	93.5	76.7	100.7	94.4	96.9
	Decreasing water	118.9	100.8	92.9	91.6	102.9	96.6	100.6
Sucrose	Control	104.9	60.4	78.1	48.5	77.9	80.4	75.0
	Decreasing water	110.4	75.4	68.8	64.7	87.3	75.5	80.4
Fructose	Control	12.6	38.5	15.4	28.2	22.8	14.2	21.9
	Decreasing water	8.5	24.0	24.2	26.9	15.7	17.8	19.5
Total reducing sugars	Control	32.8	43.7	27.9	32.9	32.7	22.8	32.1
	Decreasing water	31.8	29.8	33.3	31.2	35.7	35.4	32.9
Glucose	Control	20.2	5.2	12.5	4.7	9.9	8.6	10.2
	Decreasing water	23.2	5.9	9.1	4.3	19.9	17.7	13.4
Mean		58.1	48.2	45.6	41.0	50.6	46.3	

[1] Each figure represents the computed mean of 6 replicates.
[2] Each sugar is expressed as milligrams per gram dry weight.

Nevertheless, it is important that a measurable relationship of any kind exists between starch phosphorylase and sucrose. One question immediately arises: Suppose we possessed a treatment which would retard or block the normal starch-phosphorylase reaction. Would this then make possible a greater than normal production of sucrose? Again, what characteristics of varieties such as Uba permit these plants to form starch in quantities greater than those realized by other cane varieties? More experiments with additional treatments and other varieties must be conducted before the full nature and potential of the starch-phosphorylase reaction is understood.

AMYLASE *VS.* STARCH PHOSPHORYLASE AND SUCROSE CONTENT

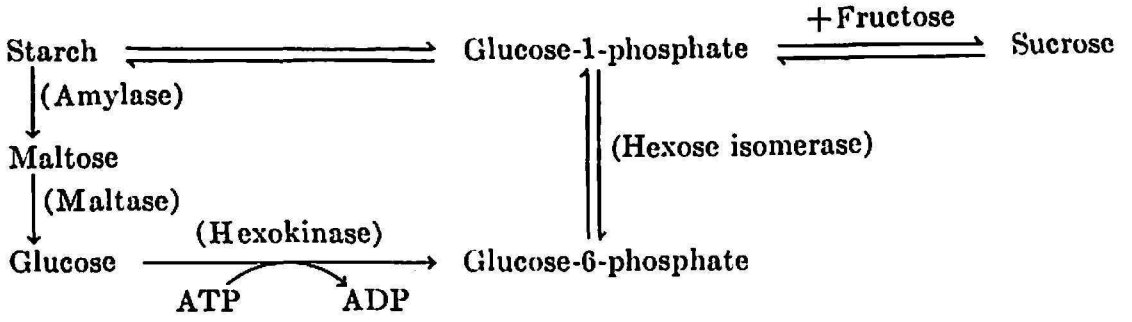
During a previous study (*1*), we observed a marked suppression of amylase by the same treatments which induced high sucrose. Grand mean

TABLE 4.—*Mean specific-activity values for leaf enzymes of immature sugarcane subjected to variable water supply in sand culture*[1]

Item No.	Enzyme	Treatment	Results at days indicated following initial treatment						Mean
			−14	0	14	28	42	56	
1	β-Glycero phosphatase	Control	9.9	41.6	26.0	32.9	50.1	50.6	35.2
		Decreasing water	9.2	30.9	38.3	30.9	23.9	30.3	27.3
2	Glucose-1-phosphatase	Control	4.2	8.2	8.0	7.6	11.5	13.1	8.8
		Decreasing water	4.1	9.1	7.5	7.6	9.5	7.1	7.5
3	ATP-ase	Control	8.5	38.3	34.4	33.8	54.6	49.7	36.6
		Decreasing water	8.1	26.6	35.3	29.8	28.4	27.0	25.9
4	Hexokinase	Control	11.6	6.2	4.5	7.9	10.9	13.2	9.1
		Decreasing water	9.2	9.1	3.6	8.7	6.1	5.8	7.1
5	Amylase	Control	46.7	108.5	58.5	69.3	111.7	78.6	79.8
		Decreasing water	28.1	58.9	70.4	83.3	75.3	48.5	60.8
6	Starch phosphorylase	Control	6.7	4.4	9.4	6.3	17.2	7.7	8.6
		Decreasing water	8.4	11.3	8.5	11.2	11.1	7.6	9.7
7	Invertase	Control	8.5	10.9	10.2	9.5	8.0	10.7	9.6
		Decreasing water	8.4	9.0	8.4	6.8	7.1	6.8	7.8
8	Sucrose phosphorylase	Control	1.05	1.44	.95	1.15	.87	1.55	1.17
		Decreasing water	.32	1.52	.70	.99	1.44	1.11	1.01
9	Peroxidase	Control	4.3	12.3	44.8	6.6	6.3	4.2	13.1
		Decreasing water	4.5	9.8	12.7	13.5	42.9	4.3	14.6
	Mean		10.0	22.2	21.2	20.4	26.5	20.4	

[1] Each figure represents the computed mean of 6 replicates. All enzymes are expressed as specific-activity values, with the exception of sucrose phosphorylase, and the latter is expressed as μmoles per milliliter of digest.

values from the present study support this observation (table 2, item 5). While summarizing the earlier work, we suggested that starch served as a source of glucose for sucrose formation, via the following amylase-maltase-hexokinase-isomerase sequence:



The proposal had a major shortcoming, however, in that the key enzyme of the sequence, amylase, was retarded rather than stimulated among plants containing high sucrose. The importance of starch was nevertheless recognized and the present study permitted us to analyze more thoroughly the amylase-sucrose relationship.

Two distinct possibilities exist concerning the low amylase-high sucrose observations. The relationship could be a direct one, that is, with suppressed amylase causing greater starch accumulation, thereby shifting the starch-forming equilibrium toward phosphorolysis and permitting more favorable accumulation of glucose-1-phosphate for sucrose synthesis. To support this trend we would look for a decline of hexokinase and such enzymes as ATP-ase and glucose-1-phosphatase. The second possibility would give the amylase suppression a secondary or indirect role, and would simply indicate that a more dominant system was utilizing glucose-1-phosphate for the production of sucrose rather than starch. The decline of amylase would then reflect a lessened need by the plant for a starch-hydrolyzing system.

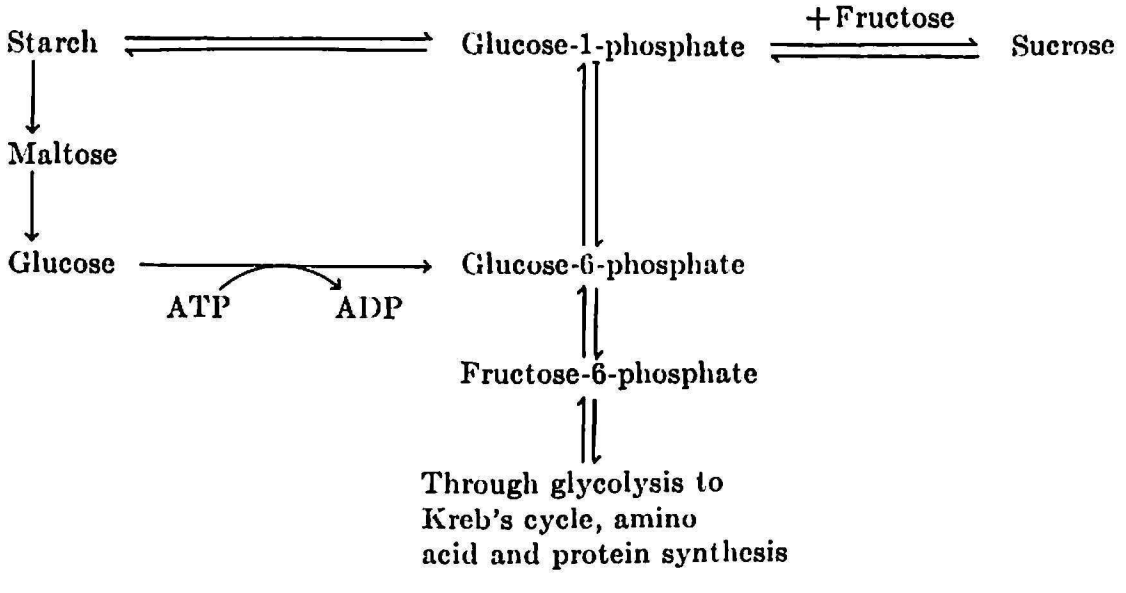
Data from the nitrate and potassium experiments show that high amylase and starch-phosphorylase activity went generally hand-in-hand, and supports the first possibility mentioned above that the counter reactions, suppressed amylase and phosphorylase, reflect a greater supply of glucose-1-phosphate for sucrose synthesis. Reviewing the amylase and starch-phosphorylase activity curves (table 2, items 5 and 6), it is evident that amylase achieved high activity at 7 days and again at 49 days among control plants of the low-nitrate experiment. Starch phosphorylase was high at 14 days rather than 7, and again at 49 days. Among control plants of the low-potassium study, we see that amylase was high at 7 and 14 days, and again at 49 days. Starch phorphorylase reached a relatively high level at 21 days and a peak at 49 days. The timelag observed between amylase and starch phosphorylase early in both experiments is interesting in that it shows an increased capacity by the plant to hydrolyze starch before the increased starch could have appeared.

Reviewing the amylase-starch phosphorylase curves for the low-water study (table 4, items 5 and 6), we see that amylase was high at 0 and 42 days. Starch phosphorylase was high at 14 and 42 days, exemplifying the

same enzyme and time relationships observed among the plants receiving low nitrate and low potassium.

It must be pointed out that the amylase-starch phosphorylase relationships discussed above, which in themselves represented extremes within the overall enzyme curves, were usually measured at 7 or 14 days following the corresponding extremes in the respective sucrose curves. This timelag, like those discussed above in which effects seem to occur before causes, is at first perplexing, and, if the reactions thus far discussed were the only ones we had to consider in cane, this behavior would be inexcusable. We should remember, however, that these plants were still relatively young (4 to 6 months) and making rapid growth. Therefore, the additional glucose-1-phosphate made available by suppressed amylase, and a starch-phosphorylase equilibrium shifted to the right, might not necessarily have entered the sucrose-phosphorylase reaction, albeit this reaction was only one enzymatic step away.

Many enzymatic steps away, far down the pathway of glycolysis and within the Kreb's cycle, there were reactions the pressing concern of which appeared to be the provision of ATP and organic acids needed for protein synthesis by growing tissues. These reactions might well have regarded a new supply of glucose-1-phosphate with more than casual interest. In other words, much of the glucose-1-phosphate which might have entered sucrose in an older plant must have been metabolized by the young plants of the present studies. Thus, while the potential for sucrose formation would include increased supply of glucose-1-phosphate, the actual stimulation of amylase and starch phosphorylase following sucrose extremes could be regarded as part of a more demanding drain upon glucose-1-phosphate, and one which permitted only a fraction of the sucrose potential to be realized. These hypothetical pathways deciding the fate of glucose-1-phosphate can be diagramed as follows:



A PROPOSED ROLE OF AMYLASE IN SUGARCANE

Up to this point we have not discussed the role of amylase in sugarcane, yet this is a question of considerable importance. Our own studies with leaf-enzyme preparations have so consistently shown the presence of this enzyme that we have come to take a powerful amylase for granted. Other systems may be weak, strong, or absent, depending upon age of the plant and experimental treatments, but amylase has invariably been present and highly active. Yet there would be no purpose for this enzyme in the absence of starch, and with the exception of Uba (*15*), starch has seldom been a quantitatively important factor in cane. The present studies have shown that the M.336 variety has a strong potential to form starch in its leaves, and yet repeated tests for starch in leaf and sheath extracts have invariably been negative.

We have come to feel, therefore, that the primary role of amylase in cane is to make sure that starch does not accumulate in more than minute quantities. It is an effective insurance that sucrose shall be the dominant storage form of excess hexoses. Sugarcane, possibly more so than most plants, has a definite need to metabolize glucose during its early months of growth and development, and the plant may not be able to afford the loss of glucose into a second major storage form. We have seen that the starch-phosphorylase reaction will proceed very well in cane-leaf preparations. But so long as a strong amylase is present, starch will be only an instantaneous factor, and a given molecule of glucose drawn into the system should pass through starch to maltose, to free glucose, to glucose-6-phosphate, and back either into glycolysis or glucose-1-phosphate in an instant of time.

Perhaps the glucose molecule in question will again be drawn through the starch system before chance places it in a more useful area. But the important point is that a strong amylase system will make certain that the glucose molecule has repeated chances for more useful employment.

On the other hand, limitations of such an insurance system are readily apparent. If something should happen to one or more of the necessary enzymes, then the glucose molecule once taken into starch might not come back at all. This is likely the case for Uba. Possibly an overly enthusiastic set of enzymes might send glucose back with a one-way ticket through glycolysis. This likely would be desirable from the plant's viewpoint. A third possibility is that the glucose could be short-circuited back into the glucose-1-phosphate pool from whence it came, and in so doing become once again eligible for the sucrose-forming reactions operating out of the same pool. It is this latter event which we believe happens, albeit inadvertently, when we withhold nitrate or water from the plant and later observe a combination of depressed amylase and increased sucrose.

An exaggerated example of the reshuffling of glucose appears to have been taking place during the final weeks of the low-potassium study. It has long been suspected that a relationship exists between potassium deficiency and sugar movements in plants. During a previous study at this Station (*1*), glucose accumulation in leaves and sheaths of cane was attributed to a suppression of the enzymes phosphohexose isomerase and phosphoglyceryl kinase during glycolysis. Data from the present study, summarized in table 1, indicate that glucose had increased in the leaves of low-potassium plants by the 49- and 63-day harvests.

A review of the enzymes summarized in table 2 reveals that starch phosphorylase, amylase, and hexokinase were all excessively active during this period. At the 63-day harvest, even the phosphatases were no longer suppressed (table 2, items 1, 2, and 3), suggesting that an unusually heavy supply of phosphorylated hexose had created a need for increased phosphatase activity. The phosphatase stimulation probably prevented any outstanding sucrose increases, although sucrose content was higher in treated plants than in controls.

SUMMARY

Four-month-old sugarcane plants were subjected to low levels of nitrate, potassium, and water in sand culture to induce variable sugar levels. Leaf samples were frozen for enzyme assay at intervals of 7 or 14 days following treatment. The objective was to study interrelationships of enzymes responsible for the synthesis and hydrolysis of sucrose, starch, and glucose phosphate. Enzymes measured included sucrose phosphorylase, starch phosphorylase, invertase, amylase, hexokinase, peroxidase, and the phosphatases hydrolyzing β-glycerophosphate, glucose-1-phosphate, and ATP.

A slight but generally consistent increase of sucrose was obtained by each of the three treatments. Sucrose phosphorylase and invertase were unaffected by any of the treatments, which placed the causes of sucrose changes upon systems less closely related to sucrose level. Phosphatases and amylase were generally suppressed by every treatment. Both the high and low extremes of sucrose content were followed, after 7 or 14 days, by starch-phosphorylase changes which were inversely related to the sucrose levels. Preliminary evidence suggests that starch phosphorylase fluctuated as a result, rather than as the cause, of sucrose changes. Availability of glucose-1-phosphate seems to have been the key factor for both the sucrose- and starch-forming systems.

Periods of high amylase activity were accompanied, or followed closely by concurrently high starch-phosphorylase activity. No starch was detected in either leaf or sheath extracts. The role of amylase in cane is

discussed in detail, particularly as a means of preventing the loss of glucose from pathways of active metabolism, or from sucrose formation.

Hexokinase varied independently of sucrose level. The enzyme was particularly active among low-potassium plants at the periods when glucose was abnormally high.

RESUMEN

A plantas de caña de azúcar de cuatro meses de edad, sembradas en arena, se les aplicaron niveles bajos de nitrato, potasio y agua, con el fin de inducir la formación de distintas cantidades de azúcar. Se congelaron algunas hojas, para efectuar análisis enzimáticos, a intervalos de 7 ó 14 días después del tratamiento. El objetivo era estudiar la interrelación de las enzimas responsables de la síntesis e hidrólisis de la sacarosa, el almidón y el fosfato de glucosa. Las enzimas que se analizaron incluían fosforilasa de sacarosa, fosforilasa de almidón, invertasa, amilasa, hexoquinasa, peroxidasa y las fosfatasas hidrolizadoras, a saber, β-glicerofosfato, fosfato glucosado -1 y ATP.

En cada uno de los tres tratamientos se obtuvo un ligero, aunque generalmente consistente, aumento en sacarosa. A la fosforilasa de sacarosa y a la invertasa no las afectó ninguno de los tratamientos, lo cual reveló que las causas de los cambios en la sacarosa dependían de sistemas menos estrechamente relacionados con el nivel de la sacarosa. Las fosfatasas y la amilasa quedaron generalmente suprimidas por cada tratamiento. Después de 7 ó 14 días, tanto al alto como al bajo contenido de sacarosa le sucedieron cambios en el contenido de la fosforilasa de almidón relacionados, inversamente, con los niveles de sacarosa. La evidencia preliminar sugiere que el contenido de fosforilasa de almidón fluctuó como resultado, antes que a causa de los cambios en la sacarosa. Tal parece que la presencia del fosfato glucosado-1 fue el factor principal de los sistemas formadores de sacarosa y almidón.

Registráronse períodos de una intensa actividad de la amilasa que estuvieron acompañados concurrentemente o seguidos muy de cerca, de una intensa actividad de la fosforilasa de almidón. No se halló almidón en los extractos tomados de las hojas o las vainas.

Discútese en detalle la importancia de la amilasa en la caña, particularmente como medio de evitar la pérdida de glucosa por "los caminos" del metabolismo activo, o por la formación de sacarosa.

El contenido de hexoquinasa varió independientemente del contenido de sacarosa. La enzima estuvo particularmente activa en las plantas con baja cantidad de potasio en períodos cuando el contenido de glucosa fue anormalmente alto.

LITERATURE CITED

1. Alexander, A. G., Sucrose-enzyme relationships in immature sugarcane as affected by varying levels of nitrate and potassium supplied in sand culture, *J. Agr. Univ. P.R. 48*(3): 165–231, 1964.
2. ——, 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, *J. Agr. Univ. P.R. 49*(1): 1–34, 1965.
3. Alexander, A. G., and González-Ibáñez, J., Residual effects of Diuron (3-[3,4-dichlorophenyl]-1,1-dimethylurea) on the sugar content and sugar-metabolizing enzymes of ratoon sugarcane 25 months following variable soil treatment, *J. Agr. Univ. P.R., 48*(4): 284–303, 1964.
4. Alexander, A. G., The biosynthesis of sucrose in sugarcane, *J. Agr. Univ. P.R. 48*(4): 265–83, 1964.
5. Balch, R. T., Further notes on starch in Louisiana canes, juices and raw sugars, *Sugar J. 15*(8): 11–15, 1953.
6. Boyes, P. M., Quantitative determinations of some non-sugars and the partial removal of one in particular—starch, Proc. S. A. Sugar Technol. Ass'n. (32nd Ann. Congress): 37–43, 1958.
7. Cardini, C. E., Leloir, L. F., and Chiriboga, J., The biosynthesis of sucrose, *J. Biol. Chem. 214:* 149–55, 1955.
8. Feuilherade, L., Starch in Uba juices, *S. A. S. J. 13*(5): 305–9, 1929.
9. Hanes, C. S., Reversible formation of starch from glucose-1-phosphate catalyzed by potato phosphorylase, *Proc. Roy. Soc.* (London) *B-129:* 174–208, 1940.
10. Hartt, C. E., The fluctuations of sugars in the leaf sheaths of the sugarcane plant during the day and the night, *Hawaii Planters Rec. 40:* 329–354, 1936.
11. Hassid, W. Z., Doudoroff, M., Barker, H. A., and Dore, W. H., Isolation and structure of an enzymatically synthesized crystalline disaccharide d-glucoside-d-ketoxyloside, *J. Amer. Chem. Soc. 68:* 1465–7, 1946.
12. Leloir, L. F., and Cardini, C. E., The biosynthesis of sucrose phosphate, *J. Biol. Chem. 214:* 157–65, 1955.
13. Lozano, B. A., and Bonner, J., Experiments on sucrose formation by potato tubers as influenced by temperature, *Plant Physiol. 24:* 720–38, 1949.
14. Paladini, A. C., and Leloir, L. F., Studies on uridine diphosphate glucose, *Biochem. J. 51:* 426–32, 1952.
15. Wood, G. G., Some factors influencing starch in sugarcane, Proc. S. African Sugar Technol. Ass'n. 123–35, April 1962.