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Behavior of Enzymes Governing Starch- and Sucrose-Forming Pathways in Two Sugarcane Varieties Supplied with Variable Nitrate and Phosphate in Sand Culture

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INTRODUCTION

Following earlier investigations which revealed a correlation between suppressed amylase and high sucrose in sugarcane (*1*)[2], more recent studies have shown relationships between the sucrose level and the enzymes starch phosphorylase, amylase, and hexokinase (*2*). It was proposed that the critical factor was glucose-1-phosphate, and the capacity of this compound to enter by either of the following pathways:

$$\text{Starch} \xrightleftharpoons{+\ H_3PO_4} \text{Glucose-1-phosphate} \xrightleftharpoons{+\ \text{Fructose}} \text{Sucrose}$$

$$\text{Glucose-1-phosphate} \rightleftharpoons \text{Glucose-6-phosphate} \rightleftharpoons \text{Glycolysis}$$

The evidence was inconclusive, however, primarily because the treatments did not give us consistently outstanding sucrose variations which could be studied from an enzymatic standpoint. The present study was designed specifically to cause more extreme sucrose responses by altering the enzyme starch phosphorylase.

It is generally agreed that the synthetic function of starch phosphorylase is to catalyze the addition of a glucose radical to the nonreducing end of a straight-chain polysaccharide, and thereby form a new α, 1-4 linkage[3]. The equilibrium constant of the reaction is determined by the molar con-

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[2] Italic numbers in parentheses refer to Literature Cited, pp. 174–5.

[3] An excellent review of the evidence favoring this concept has been summarized by Peat (*18*).

centrations of the polysaccharide, of glucose-1-phosphate, and of inorganic phosphate (*9*, p. 166):

$$Keq = \frac{(C_6H_{10}O_5)n\text{-}1\ (\text{Glucose-1-}PO_4)}{(C_6H_{10}O_5)n\ (H_3PO_4)}$$

However, the **effective** polysaccharide concentration represents the number of nonreducing polymer termini, a number that does not change, and therefore the molar concentration of the polysaccharide may be regarded as a constant. It follows that at a given pH, the *Keq* will be determined by relative proportions of glucose-1-phosphate and inorganic phosphate:

$$Keq = \frac{(\text{Glucose-1-}PO_4)}{(H_3PO_4)}$$

It is evident that the inorganic phosphate levels within a plant would conceivably affect the starch phosphorylase equilibrium. Being readily within our means, this was one of the variables selected for the present study.

The pH of the endogenous reaction was another factor we attempted to control as a variable. It is clear from the above equilibrium constants that several ionic species could react with the enzyme, including orthophosphate as $(HPO_4)^{--}$ or $(H_2PO_4)^{-}$, and glucose-1-phosphate as $(C_6H_{11}O_5 \cdot O \cdot PO_3)^{--}$ or $(C_6H_{11}O_5 \cdot O \cdot PO_3H)^{-}$. Hanes (*16*), working with partly purified starch phosphorylase from potato, concluded that the reaction proceeds in either direction until the ratio of mineral phosphorus to organic phosphorus achieves a value not altered by variable starch, mineral phosphate, or organic phosphate concentrations:

$$\begin{array}{ccc} \text{Starch} + (HPO_4)^{--} & \rightleftharpoons & (C_6H_{11}O_5 \cdot O \cdot PO_3)^{--} \\ \downarrow\uparrow & & \downarrow\uparrow H^+ \\ (H_2PO_4)^{-} & & (C_6H_{11}O_5 \cdot O \cdot PO_3H)^{-} \end{array}$$

At equilibrium the value of this ratio will depend on hydrogen-ion concentration. Thus, the ratio of total mineral phosphate to total glucose-1-phosphate was 10.8 at pH 5, and 3.1 at pH 7. Cori and Cori (*11*), working with muscle phosphorylase and glycogen, reported similar pH effects. The ratio of mineral phosphorus to ester phosphorus was 5.7 at pH 6.0, and 2.7 at pH 7.6.

In order to induce variable acidity within the plants of the present study, high and low levels of nitrate were supplied. Vickery (*26*) found that the organic acid content of tobacco plants was many times greater when

nitrogen was supplied as nitrate rather than as ammonia. Similar nitrate-organic acid relationships have been reported in the tomato (*8*) and other plant species (*19,27*).

A third treatment involved the use of two sugarcane varieties which varied distinctly in their reputed ability to form sucrose and starch. The M.336 variety has long been considered a very "sweet", high-sucrose producer by workers in Puerto Rico. The Uba variety, no longer extensively grown in Puerto Rico, has a somewhat dubious reputation for producing unusually large quantities of starch (*25,28,29*).

MATERIALS AND METHODS

One-eye cuttings of the varieties M.336 and Uba Marot were planted in quartz sand on November 25, 1963, and all seedlings received distilled water until treatments were initiated on December 24, 1963. The sand had been treated previously with 0.05 N HCl for 12 hours, and after thorough leaching with tapwater it was placed in 5-gallon, polyethylene containers fitted with glass wool over the drainage outlets. The sand was then leached with distilled water before receiving the cuttings.

The seedlings first received nutrient solutions when they were 4 weeks of age. Each container was supplied daily with 1 liter of nutrient solution at 8 a.m., and 1 liter of distilled water at 1 p.m., until the study was terminated at 12 weeks. Nitrate was provided at 12.0 and 1.5 meq./liter, and phosphate at 6.0 and 0 meq./liter.

All other essential nutrients were maintained at constant levels[4]. The treatments therefore consisted of two varieties, each receiving two levels of nitrate and phosphate. A $2 \times 2 \times 2$ factorial design was employed with four replicates for each of the eight treatments.

Tissue samples were frozen for sugar and enzyme assay at the close of the study. The samples consisted of meristematic tissue, and of leaves +1 to +5[5] from the four most uniform plants of each container. The plant material was frozen in a mixture of Dry Ice (solid CO_2) and acetone, lyophilized, and extracted by procedures described previously in detail (*1*). Enzyme preparations consisted of dialyzed, water-soluble protein, precipitated from extracts by 95-percent saturation with ammonium sulfate at 20°C. and pH 7.0. Protein was measured colorimetrically by the micromethod of Sutherland *et al.* (*23*).

[4] These nutrients, expressed as milliequivalents per liter, were supplied as follows: Potassium, 5.0; calcium, 3.0; magnesium, 2.0; and sulfate, 2.0. Micronutrients, 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.

[5] We employ the system of leaf nomenclature by which the uppermost leaf bearing a visible dewlap is designated +1.

Total ketose was determined by the resorcinol method of Roe (*21*). Sucrose was measured according to the modification of Cardini *et al.* (*7*), by which fructose is destroyed through heating the samples in the presence of 0.25 *N* NaOH. Total reducing sugars were measured by the dinitrosalycilic acid technique of Sumner (*22*). Details of the protein and sugar analyses have been described previously (*1*).

Enzymes assayed during these investigations included sucrose phosphorylase, starch phosphorylase, invertase, amylase, hexokinase, peroxi-

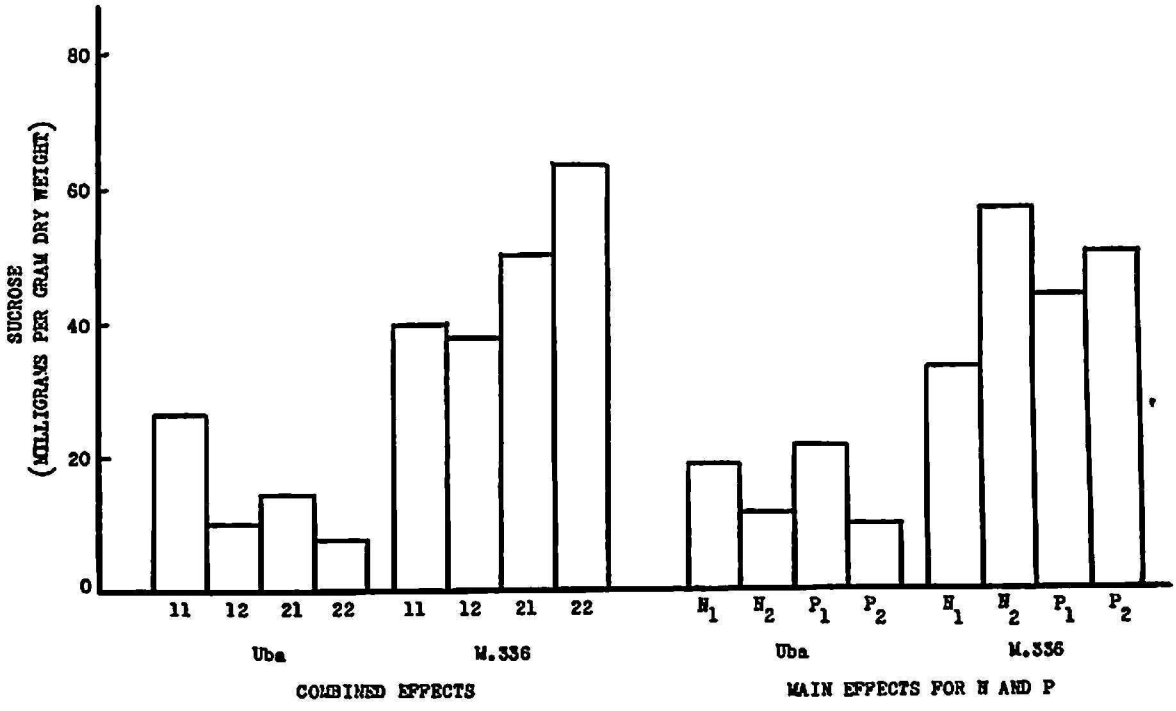


FIG. 1.—Combined and main effects for meristem sucrose values of Uba and M 336. The numerals 1 and 2 refer to the following nitrate and phosphate combinations: 11 = low nitrate, low phosphate; 12 = low nitrate, high phosphate; 21 = high nitrate, low phosphate; and 22 = high nitrate, high phosphate. Nitrate was supplied at rates of 1.5 and 12.0 meq./liter, and phosphate at 0 and 6.0 meq./liter.

dase, polyphenol oxidase, cytochrome-C reductase, and the phosphatases catalyzing the hydrolysis of β-glycerophosphate, ATP, ADP, glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, and 3-phosphoglyceric acid. With but minor changes in technique, all assays have been thoroughly described in the earlier paper (*1*), except sucrose phosphorylase (*4*) and starch phosphorylase (*2*). ADP-ase was measured in identical manner as ATP-ase. Cytochrome-C reductase could not be detected in the meristem preparations.

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

RESULTS AND DISCUSSION

TREATMENTS *vs.* SUCROSE CONTENT OF LEAF AND MERISTEM

Leaf sucrose did not vary appreciably, but a broad range of sucrose differences was found among meristem samples for each of the three treat-

TABLE 1.—*Leaf and meristem sugars from 2 varieties of sugarcane supplied with variable nitrate and phosphate in sand culture*[1]

Treatment[2]				Total ketose	Fructose	Sucrose	Total reducing	Glucose	Mean
No.	Var. × NO_3 × PO_4								
Leaf Sugars									
1	Uba	1	1	56.6	11.5	45.1	22.7	11.2	29.4
2	Uba	1	2	52.1	9.9	42.2	23.3	13.4	28.2
3	Uba	2	1	66.8	18.1	48.8	24.8	6.7	33.0
4	Uba	2	2	63.5	16.1	47.4	22.1	6.3	31.1
5	M.336	1	1	52.6	9.8	42.8	24.1	14.3	28.7
6	M.336	1	2	53.1	8.2	44.9	21.6	13.5	28.3
7	M.336	2	1	62.2	9.4	54.6	17.5	8.3	30.4
8	M.336	2	2	67.7	12.2	55.5	18.2	8.0	32.3
Means for leaf sugars				59.3	11.9	47.7	21.8	10.2	30.2
Meristem sugars									
1	Uba	1	1	202.3	175.8	26.5	186.5	10.6	120.3
2	Uba	1	2	150.1	140.0	10.1	150.1	10.0	92.1
3	Uba	2	1	214.7	201.6	14.2	219.8	16.9	133.4
4	Uba	2	2	224.6	216.7	7.9	238.5	20.8	141.7
5	M.336	1	1	197.6	158.9	38.6	180.9	22.0	119.6
6	M.336	1	2	191.8	154.7	37.1	176.5	21.8	116.4
7	M.336	2	1	223.9	184.2	49.2	215.1	30.8	140.6
8	M.336	2	2	245.7	181.9	63.8	217.1	35.2	148.7
Means for meristem sugars				206.3	176.7	30.9	198.1	21.0	126.6

[1] Each figure represents the computed mean of 4 replicates. Sugars are expressed as milligrams per gram dry weight.

[2] The treatment numerals 1 and 2 refer to the nitrate levels of 1.5 and 12.0 meq./liter, respectively, and to the phosphate variables of 0 and 6.0 meq./liter, respectively.

ments (table 1, fig. 1). This was precisely the type of sugar response we had been seeking, and meristems will therefore receive primary attention throughout this discussion.

Sucrose was significantly lower in Uba meristems than in those of M.336. Nitrate and phosphate effects were also expressed according to the variety being tested. High nitrate retarded sucrose in Uba and promoted sucrose in M.336 (fig. 1, main effects). High phosphate greatly depressed sucrose in Uba, but slightly increased the sugar level in M.336. It is interesting to note that the optimum treatment for sucrose in M.336, a combination of high nitrate and high phosphate, was easily the least productive treatment for Uba. The capacity of phosphate to alter the sucrose level depended both upon variety and nitrate level. Thus, high phosphate severely depressed sugar content at the low-nitrate level in Uba, but it had no effect at the low-nitrate level on M.336, and increased sucrose at the high-nitrate level in M.336 (fig. 1, combined effects).

SUCROSE CONTENT *vs.* ENZYME ACTIVITY IN MERISTEM

Enzymes were generally far more active in Uba than M.336. Grand mean values presented in table 2 indicate that enzyme activity in M.336 was about 60 percent of that recorded for Uba, but enzyme suppression varied greatly among individual systems. Among the acid phosphatases, ADP-ase and ATP-ase were particularly suppressed in M.336, while glucose-1-phosphatase was only moderately affected. It is perhaps significant that the only phosphatase not found to be retarded in M.336 was the enzyme hydrolyzing 3-phosphoglyceric acid, a compound farthest removed from the series of organic phosphates biochemically aligned about the starch-forming mechanism under scrutiny. Amylase, like the phosphatases, was suppressed by the M.336 treatment in general. Amylase and phosphatase were both inversely related to sucrose level in meristems (table 1, fig. 2). Hexokinase was far less active in M.336 than Uba, and this difference was almost entirely confined to the plants receiving low nitrate.

The enzyme most singularly affected by variety differences was invertase. In M.336 this system was active in the range of 20 to 30 percent of the Uba invertase, and this reaction in itself may account for much of the Uba-M.336 discrepancy in meristem sucrose. Invertase has not been of consequence, however, in any of our studies with leaf preparations. High phosphate appeared to stimulate the enzyme in Uba, but not in M.336.

Generally speaking, it appears that variety differences, exemplified by a definite slackening of enzyme activity on the part of M.336, were of paramount importance in determing the respective sucrose levels of meristematic tissues. Nitrate and phosphate, undeniably powerful forces in shaping the physiological welfare of cane, usually served to alter the degree, rather than the direction, of overall sucrose and enzyme responses. Nevertheless, the behavior of enzymes under all the conditions prescribed by this study merits further analysis in detail.

TABLE 2.—*Mean specific-activity values for meristem enzymes from 2 varieties of sugarcane supplied with variable nitrate and phosphate in sand culture*[1]

Item No.	Enzyme[3]	Treatment[2]										Individual means								Grand means			
		Uba N_1P_1	Uba N_1P_2	Uba N_2P_1	Uba N_2P_2	Uba mean	M.336 N_1P_1	M.336 N_1P_2	M.336 N_2P_1	M.336 N_2P_2	M.336 mean	Uba N_1	Uba N_2	Uba P_1	Uba P_2	M.336 N_1	M.336 N_2	M.336 P_1	M.336 P_2	N_1	N_2	P_1	P_2
1	β-glycerophosphatase	48.8	54.5	43.3	43.1	47.4	37.4	39.7	38.3	39.6	38.8	51.6	43.2	46.0	48.8	38.5	38.9	37.8	39.6	44.7	41.1	41.9	44.2
2	ATP-ase	40.9	54.2	45.2	42.3	45.7	30.1	31.6	30.3	29.5	30.4	47.5	43.7	43.0	48.3	30.8	29.9	30.2	30.5	39.2	36.8	36.6	39.4
3	ADP-ase	41.9	47.3	39.6	39.3	42.0	23.4	24.1	23.5	23.2	23.7	44.6	39.5	40.7	43.3	23.7	23.3	23.4	23.6	34.2	31.4	32.1	33.5
4	Glucose-1-phosphatase	19.0	21.5	16.9	17.0	18.6	11.4	13.4	10.3	10.9	11.5	20.2	16.9	17.9	19.2	12.4	10.6	10.8	12.1	16.3	13.8	14.4	15.7
5	Glucose-6-phosphatase	10.5	13.6	10.3	11.1	11.38	7.9	8.9	7.8	8.3	8.2	12.0	10.7	10.4	12.3	8.4	8.0	7.8	8.6	16.3	9.4	9.1	10.5
6	Fructose-6-phosphatase	24.9	18.8	14.2	15.3	18.3	11.5	12.2	9.4	8.8	10.5	21.8	14.7	19.5	17.0	11.8	9.1	10.4	10.5	16.9	11.9	15.0	13.8
7	3 PGA-ase	24.0	24.2	16.9	18.8	20.9	20.6	23.0	18.1	18.9	20.2	24.1	17.8	20.4	21.5	21.8	18.5	19.3	20.9	22.9	18.2	19.9	21.2
8	Amylase	78.7	74.2	59.3	55.6	66.9	34.1	38.4	33.2	37.3	35.8	76.4	57.4	69.0	64.9	36.2	35.2	33.6	37.8	56.3	46.3	51.3	51.4
9	Starch phosphorylase	33.4	26.2	16.4	20.8	24.2	9.8	10.0	15.4	14.0	12.3	29.8	18.6	24.8	23.4	11.4	13.2	12.6	12.0	20.6	15.8	18.8	17.8
10	Invertase	26.4	33.7	27.7	39.9	31.9	8.5	10.5	8.6	7.7	8.8	30.0	33.8	27.0	36.8	9.5	8.1	8.5	9.1	19.8	20.9	17.8	22.9
11	Sucrose phosphorylase	2.25	2.17	2.28	2.28	2.25	2.32	2.09	2.22	2.43	2.27	2.21	2.28	2.26	2.22	2.20	2.33	2.27	2.26	2.21	2.30	2.27	2.24
12	Hexokinase	68.9	49.4	29.3	31.9	44.9	20.3	26.5	20.0	27.6	23.6	59.1	30.6	49.1	40.6	23.4	23.8	20.1	27.0	41.3	27.2	34.6	33.9
13	Peroxidase	10.4	11.3	12.3	10.4	11.1	12.2	12.5	13.9	14.9	13.4	10.8	11.3	11.3	10.8	12.4	14.4	13.0	13.7	11.6	12.9	12.2	12.3
14	Polyphenol oxidase	8.9	7.9	14.2	15.1	11.5	5.1	5.9	4.7	6.8	5.6	8.4	14.6	11.5	11.5	5.5	5.7	4.9	6.3	6.9	10.2	8.2	8.9
	Mean	31.4	31.3	24.9	25.9	28.4	16.9	18.6	16.7	17.8	17.5	31.4	25.4	28.1	28.6	17.7	17.2	16.8	18.1	24.9	21.3	22.6	23.5

[1] Each figure represents the computed mean of 4 replicates.

[2] The treatment numerals 1 and 2 refer to the nitrate levels of 1.5 and 12.0 meq./liter, respectively, and to the phosphate variables of 0 and 6.0 meq./liter, respectively. The capital letters N and P are substituted for the words "nitrate" and "phosphate".

[3] Each enzyme is recorded in specific-activity values (units per milligram of protein), with the exception of sucrose phosphorylase which is expressed as μmoles of sucrose formed per milliliter of digest.

SUCROSE CONTENT *vs.* THE PHOSPHORYLASES

Sucrose phosphorylase remained remarkably constant among all treatments (table 2), its product varying only from 2.09 to 2.43 μmoles of sucrose per milliliter of digest. This variation in itself cannot account for sucrose differences of the magnitude actually encountered. It does, however, add evidence to the contention expressed earlier (*2*) that sucrose variations

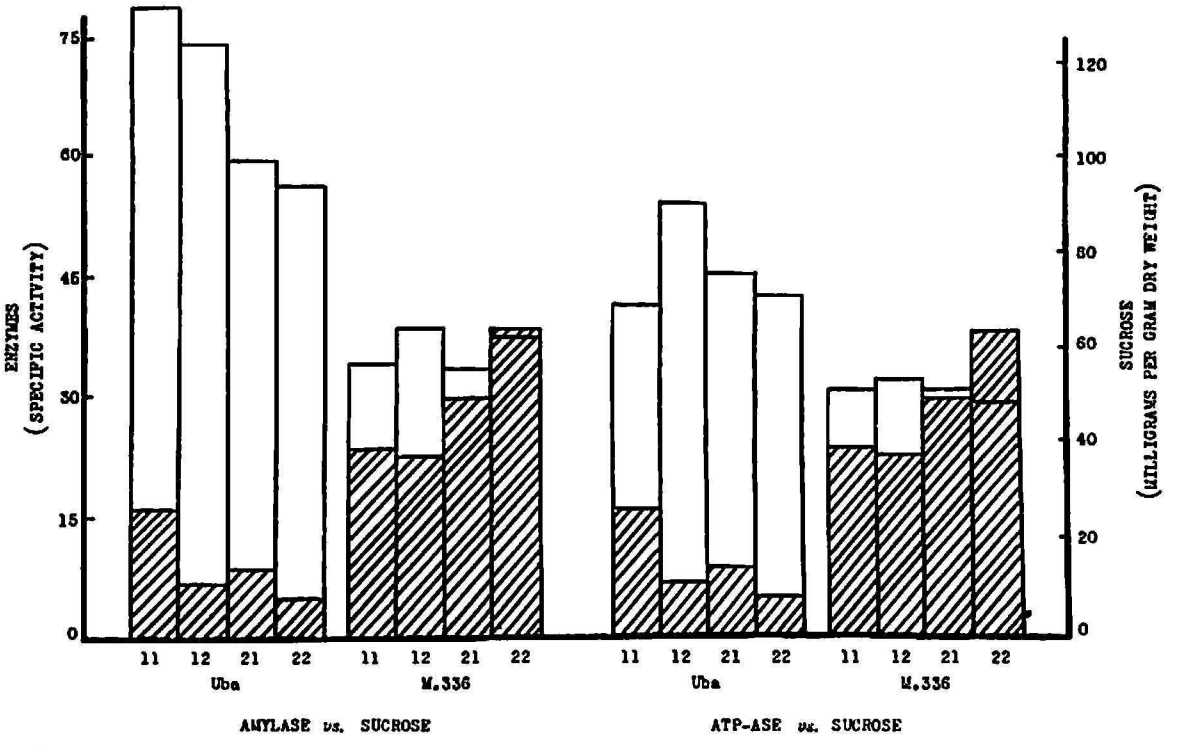


FIG. 2.—A comparison of sucrose content and enzyme activity in the meristems of Uba and M.336. The numerals 1 and 2 refer to the following nitrate and phosphate combinations: 11 = low nitrate, low phosphate; 12 = low nitrate, high phosphate; 21 = high nitrate, low phosphate; and 22 = high nitrate, high phosphate. Nitrate was supplied at rates of 1.5 and 12.0 meq./liter and phosphate at 0 and 6.0 meq./liter. The shaded areas illustrate sucrose levels which have been superimposed over the enzyme data.

may be caused less by sucrose phosphorylase than by enzymes farther removed from the reactions of sucrose synthesis.

Starch phosphorylase was about twice as active in Uba as in M.336, or in other words, the enzyme varied inversely with sucrose among variety treatments. Recalling the work of Hanes (*16*) with potato phosphorylase, we know that, at pH 7, the reaction achieves an equilibrium in which the ratio of total mineral phosphate to total glucose-1-phosphate will be about 3.1:

$$Keq = \frac{(H_3PO_4)}{(\text{Glucose-1-PO}_4)}$$

$$= 3.1_{(pH\ 7.0)}$$

The enzyme in living meristem tissue must have been acting at a pH lower than 7.0, and consequently an even higher *Keq* value than 3.1 would be anticipated. The apparent varietal effect upon this enzyme was to shift the reaction equilibrium toward the left in Uba,

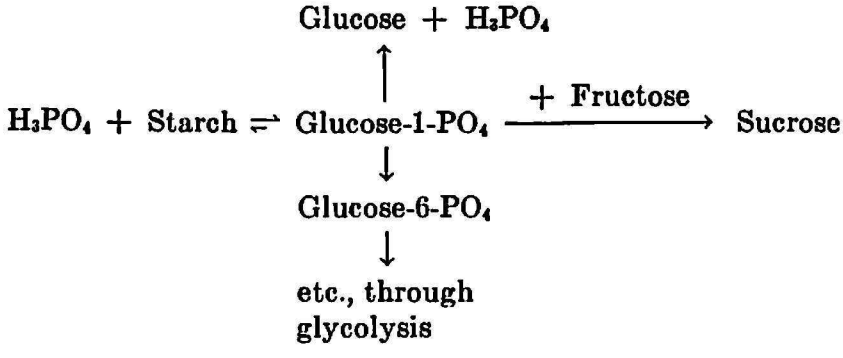
$$H_3PO_4 + \text{Starch} \longleftarrow \text{Glucose-1-PO}_4$$

and toward the right in M.336:

$$H_3PO_4 + \text{Starch} \longrightarrow \text{Glucose-1-PO}_4$$

The treatment effects must have been equivalent to altering pH, and if pH did represent the mode of varietal action, then Uba was the variety most able to assume more acid conditions. The increased product of the M.336 reaction would not be sucrose, but rather glucose-1-phosphate, an essential precursor of sucrose.

The added glucose-1-phosphate would be confronted with at least three known alternatives: 1, Direct hydrolysis by acid phosphatase; 2, conversion to sucrose; and 3, assimilation into the glycolytic pathway and eventual metabolism. These alternatives can be diagramed as follows:



Respiration was not measured during this study and we do not know to what extent glucose-1-phosphate was entering metabolic pathways. However, during the 6 weeks prior to harvest, the demand for glycolytic products must have been heavy, since this represented a period of extremely rapid growth. On the other hand, we know that glucose-1-phosphatase was not particularly active in M.336, and, in fact, it was considerably suppressed as compared with its counterpart in Uba. Since we know that much more sucrose was accumulating in the meristems of M.336 than of Uba, it appears that an increased amount of glucose-1-phosphate was passing directly into sucrose as a result of the starch-phosphorylase suppression in M.336.

The opportunity for sucrose accumulation in Uba would seem particularly hard-pressed. Uba's starch phosphorylase showed a greater preference for starch formation than for the reverse reaction, phosphorolysis, which would have provided more glucose-1-phosphate for sucrose synthesis. Uba also possessed a greater capacity to hydrolyze glucose-1-phosphate,

and it contained a voracious invertase to attack whatever sucrose might have been formed.

The nitrate and phosphate variables, which were intended to shift the starch phosphorylase equilibrium, achieved only part of the anticipated results. Variety effects, plus the dispersal of reactions among leaf and meristem tissues, tended to confuse the picture. We had considered that the plants receiving high nitrate should have contained a high enough organic acid content to increase the *Keq* value, *i.e.*, to increase the ratio of mineral phosphate to ester phosphate. The high-nitrate effect should have suppressed the plants' relative capability to produce sucrose by lowering the proportion of glucose-1-phosphate entering the sucrose phosphorylase pathway. The use of variable phosphate was intended to alter the same equilibrium. High phosphate should theoretically have shifted the reaction in favor of phosphorolysis, or in other words, should have indirectly promoted sucrose production. The hypothetical effects of nitrate and phosphate are illustrated by the following sequences:

1, High NO_3	→	increased acidity	→	increased starch-forming potential	→	decreased sucrose
2, Low NO_3	→	decreased acidity	→	decreased starch-forming potential	→	increased sucrose
3, High PO_4	→	increased phosphorolysis	→	decreased starch-forming potential	→	increased sucrose
4, Low PO_4	→	decreased phosphorolysis	→	increased starch-forming potential	→	decreased sucrose

A review of sucrose values for meristems (table 1, fig. 1) shows that the anticipated effects for nitrate held true in Uba, that is, high nitrate suppressed sucrose while low nitrate increased sucrose. Just the opposite nitrate effects were recorded for M.336. High phosphate depressed sucrose in Uba and had little effect in M.336.

Reviewing enzyme values for meristem tissues, we find that the anticipated starch phosphorylase responses to nitrate did not appear in the meristems at all (table 2, item 9), but rather in the leaves (table 3, item 9). Thus, while both the sucrose and enzyme effects anticipated for the nitrate treatments did in fact occur, they were not correlated within the same tissues. The phosphate effects we had desired apparently were not achieved. However, there are so many related factors affecting inorganic phosphate level in cane that we will discuss this point in a separate section p. 165ff.

The physical isolation of treatment responses, which, on paper, are separated by only a few enzymatic reactions, is not very surprising when one considers the multitude of related factors involved. The fact that anticipated enzyme responses and sucrose levels occurred in separate tissues may simply have been a matter of enzyme isolation and sucrose transloca-

tion. The meristem tissue in cane is not an ideal area for sugar storage, and sucrose levels beyond some minimum value must logically be moved to more favorable storage areas. In 4- to 12-week-old cane this would be the leaves. A case in point was scored by the relatively high leaf-sucrose levels encountered among high-nitrate plants of the M.336 variety (table 1). The appearance here of high sucrose ran counter to our previous observations (*1,2,3,5*), *i.e.*, it appeared where each of the acid phosphatases, plus amylase, peroxidase, and starch phosphorylase, were stimulated rather than suppressed. A large share of this sucrose must have been imported from meristematic areas.

However, since the meristem is an area of intense biochemical activity, it would also seem logical that some reactions of the sucrose-forming and metabolyzing pathways could best be carried out in the meristem. Another case in point was brought forward by the localization of invertase in the meristems of both varieties tested. Sucrose would have to pass to this area in order to be inverted, and thus a continuous translocation of sucrose to and from the meristematic area is not an unreasonable assumption.

Other factors need to be considered from the standpoint of overall nitrogen nutrition when pondering the enzyme and sugar responses of this study. We should not regard our nitrate variables with too much finality. In terms of milliequivalents per liter, we do not know exactly where the concepts of high nitrate and low nitrate can be applied. Perhaps the nitrate levels of 12.0 and 1.5 meq./liter might actually represent high and moderate levels for one variety, and moderate and deficient levels for another. Perhaps 25 or 30 meq./liter of nitrate would have better served our purpose during the present investigations. Again, it might have been more logical to supply constant nitrogen levels to all plants and simply vary the source between nitrate and ammonia. Finally, no trained physiologist will dispute the fact that there is a great difference between altering the pH for a reaction in a test tube, and doing the same for that reaction while it is still an integral part of a living plant.

A good example of high-nitrate repercussions among two distinct cane varieties, and the unintentional altering of enzymes by treatments meant for starch phosphorylase, is illustrated by the enzyme values obtained from the M.336 leaf preparations (table 3). The diagram presented by figure 3 illustrates the areas in which reactions have been greatly increased by high nitrate. Those reactions significantly stimulated are set off with heavy arrows. In each instance, the nitrate effect was a characteristic of the M.336 variety, and the same nitrate level caused no changes of consequence in Uba.

Nevertheless, with due consideration given to all suggestions peculiar to hindsight, the results of this study support the original contention that a

TABLE 3.—*Mean specific-activity values for leaf enzymes from 2 varieties of sugarcane supplied with variable nitrate and phosphate in sand culture*[1]

Item No.	Enzyme[3]	Treatments[2]										Individual means								Grand means			
		Uba N_1P_1	Uba N_1P_2	Uba N_2P_1	Uba N_2P_2	Uba mean	M.336 N_1P_1	M.336 N_1P_2	M.336 N_2P_1	M.336 N_2P_2	M.336 mean	Uba N_1	Uba N_2	Uba P_1	Uba P_2	M.336 N_1	M.336 N_2	M.336 P_1	M.336 P_2	N_1	N_2	P_1	P_2
1	β-glycerophosphatase	43.6	36.4	34.1	32.4	36.6	37.7	40.4	60.9	70.6	52.4	40.0	33.2	38.8	34.4	39.0	65.8	49.3	55.5	39.5	49.5	44.0	44.9
2	ATP-ase	54.7	42.6	49.6	39.7	46.7	42.2	42.4	70.5	80.0	58.8	48.6	44.6	52.1	41.1	42.3	75.2	56.3	61.2	45.4	59.9	54.2	51.1
3	ADP-ase	31.1	28.3	29.2	28.8	29.4	23.2	26.4	38.2	43.5	32.8	29.7	29.0	30.1	28.5	24.8	40.8	30.7	34.9	27.2	34.9	30.4	31.7
4	Glucose-1-phosphatase	9.1	8.8	10.2	10.1	9.6	7.0	8.1	9.7	10.6	8.9	8.9	10.1	9.6	9.4	7.5	10.1	8.3	9.3	8.2	10.1	8.9	9.3
5	Glucose-6-phosphatase	10.5	10.0	9.8	9.6	9.9	9.7	11.2	15.8	19.1	13.9	10.2	9.7	10.1	9.8	10.4	17.4	12.7	15.1	10.3	13.5	11.4	12.4
6	Fructose-6-phosphatase	13.3	9.0	10.1	8.6	10.3	11.5	11.0	21.1	24.7	17.1	11.1	9.3	11.7	8.8	11.2	22.9	16.3	17.8	11.1	16.1	14.0	13.3
7	3 PGA-ase	42.8	33.8	39.9	40.3	39.2	32.8	36.8	51.5	56.5	44.4	38.3	40.1	41.3	37.0	34.8	54.0	42.1	46.6	36.5	47.0	41.7	41.8
8	Amylase	46.3	40.3	45.3	52.8	46.2	42.0	38.9	68.5	87.7	59.3	43.3	49.0	45.8	46.5	40.4	78.1	55.2	63.3	41.8	63.5	50.5	54.9
9	Starch phosphorylase	10.2	9.6	12.8	12.8	11.4	8.2	7.8	16.4	19.3	12.9	9.9	12.8	11.5	11.2	8.0	17.8	12.3	13.5	8.9	15.3	11.9	12.3
10	Invertase	3.3	3.3	3.2	3.2	3.2	2.2	2.2	2.2	2.1	2.2	3.3	3.2	3.2	3.2	2.2	2.1	2.2	2.1	2.7	2.6	2.7	2.6
11	Sucrose phosphorylase	1.65	2.09	1.89	1.51	1.79	1.82	2.03	1.75	1.37	1.74	1.87	1.70	1.77	1.80	1.92	1.56	1.78	1.70	1.89	1.63	1.77	1.75
12	Hexokinase	24.1	22.7	26.3	28.1	25.3	20.0	25.1	33.7	35.4	28.6	23.4	27.2	25.2	25.4	22.5	34.5	26.8	30.2	22.9	30.8	26.0	27.8
13	Peroxidase	5.4	5.5	7.1	7.6	6.4	3.5	3.6	5.6	5.8	4.6	5.4	7.3	6.2	6.5	3.5	5.7	4.5	4.7	4.4	6.5	5.3	5.6
14	Polyphenol oxidase	19.3	19.7	22.5	23.2	21.2	15.6	17.1	19.1	20.7	18.1	19.5	22.8	20.9	21.4	16.3	19.9	17.3	18.9	17.9	21.3	19.1	20.1
15	Cytochrome-C reductase	6.0	6.8	9.2	6.3	7.1	4.7	4.2	4.8	4.3	4.5	6.4	7.7	7.6	6.5	4.4	4.5	4.7	4.2	5.4	6.1	6.1	5.3
	Mean	21.4	18.6	20.6	20.3	20.3	17.5	18.5	28.0	32.1	24.0	20.0	20.5	21.1	19.4	17.9	30.0	22.7	25.3	18.9	25.2	21.9	22.3

[1] Each figure represents the computed mean of 4 replicates.

[2] The treatment numerals 1 and 2 refer to the nitrate levels of 1.5 and 12.0 meq./liter, respectively, and to the phosphate variables of 0 and 6.0 meq./liter, respectively. The capital letters N and P are substituted for the words "nitrate" and "phosphate".

[3] Each enzyme is recorded in specific-activity values (units per milligram of protein), with the exception of sucrose phosphorylase which is expressed as μmoles of sucrose formed per milliliter of digest.

strong relationship exists in cane between starch-forming capacity and sucrose content. This relationship is further affected by the factors of nitrate supply and variety.

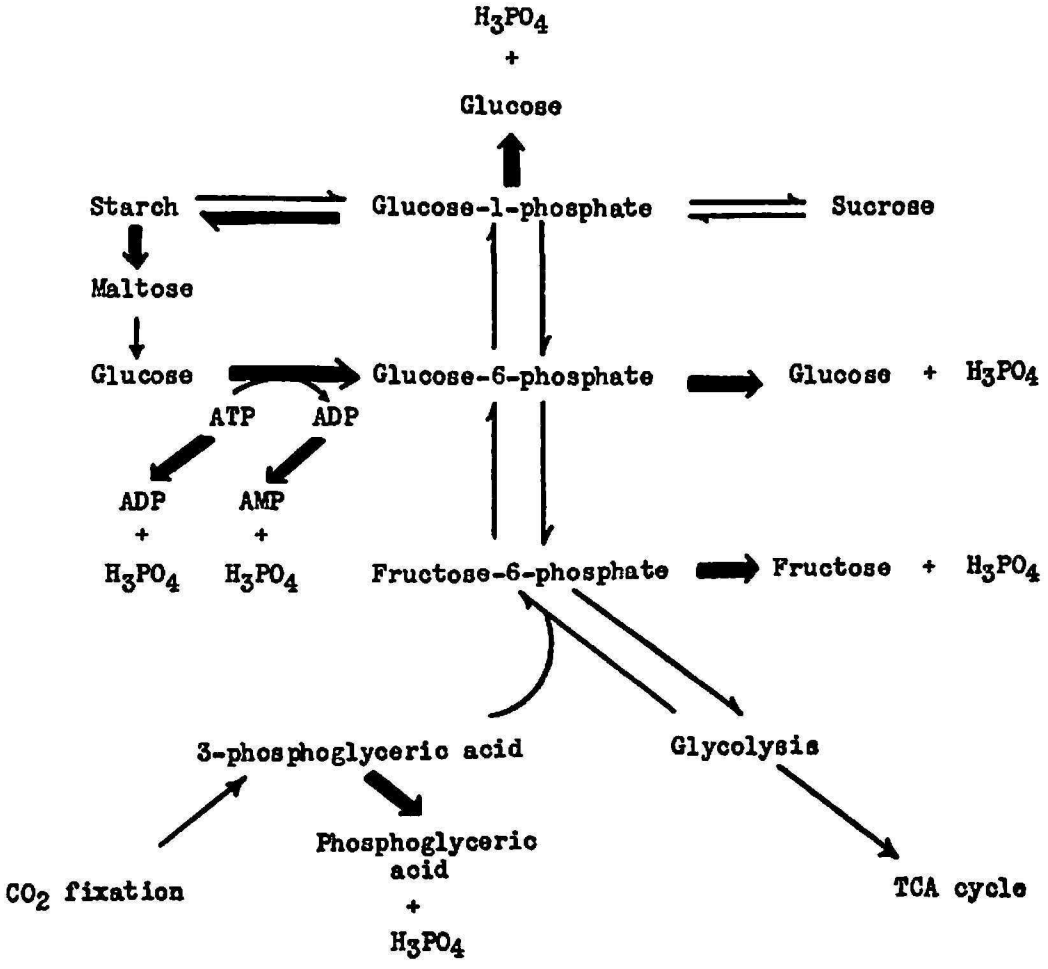


FIG. 3.—Diagramatic illustration of the biochemical pathways upon which the enzymes herein discussed are employed. In the above sequence, heavy arrows set off enzyme reactions significantly stimulated by high nitrate, as compared with low nitrate, in leaf preparations of the variety M.336.

STARCH PHOSPHORYLASE *vs.* THE PHOSPHATE NUTRITION OF SUGARCANE

A point has been reached where the concept of phosphate supply needs to be considered from a standpoint other than its effect on the H_3PO_4/glucose-1-PO_4 ratio. In particular, the overall balance between organic and inorganic phosphate in the plant appears to be somehow related to enzymes involved in starch formation and hydrolysis. This problem remains a hazy one, but enough information has been published to suggest an extremely promising area for future research.

Prior to 1935 it was incorrectly assumed that the breakdown of glycogen in animal tissues was catalyzed by amylases (*12*, p. 438). In that year,

inorganic phosphate was found to be an essential participant of glycogen degradation. Cori *et al.* (*10*) identified glucose-1-phosphate as a product of the reaction, which now was correctly termed "phosphorolysis". Within 3 years, Hanes (*16*) described a similar reaction in the potato by which starch is converted to glucose-1-phosphate.

The complexity of phosphorylase became more apparent when Green and Cori (*15*) obtained the enzyme in crystalline form from muscle. The crude enzyme had been inactive without added AMP (adenosine monophosphate), and activity of the purified enzyme was stimulated when AMP was present. The purified enzyme did not require AMP, however, and the reason for this became known when a third enzyme, presumably a proteinase, converted the crystalline enzyme (phosphorylase *a*) into a form inactive in the absence of AMP (phosphorylase *b*).

Rall *et al.* (*20*) observed similar active and inactive phosphorylase relationships, but with enzymes obtained from different animal sources. The purified, active form was converted to an inactive form by an enzyme which released inorganic phosphate during the reaction:

$$\text{Phosphorylase } a \xrightarrow[\text{Phosphatase}]{\text{Phosphorylase}} \text{Phosphorylase } b + H_3PO_4$$

The above reaction suggests that active phosphorylase is a phosphoprotein, which is inactivated by a specific phosphatase. The participation of the adenylic acid system again became evident when it was learned that ATP and a specific kinase were required to reactivate phosphorylase *b*:

$$\text{Phosphorylase } b + \text{ATP} \xrightarrow[Mg^{2+} \text{ or } Mn^{2+}]{\text{Kinase}} \text{Phosphorylase } a + \text{ADP}$$

Close similarities exist between muscle phosphorylase and the enzyme extracted from potato (*13*, pp. 443–4). Starch phosphorylase of sugarcane has not yet been thoroughly examined, but we cannot overemphasize the fact that, should the active enzyme ever be proven to exist as a phosphoprotein (like phosphorylase of muscle), then this fact would assume paramount importance in clarifying starch-sucrose relationships in cane. Our own studies have consistently shown the presence of powerful acid phosphatases in leaf preparations, and we have been particularly concerned with the hydrolysis of the glucose phosphates and ATP in relation to sucrose content. Sucrose content was significantly increased by use of acid phosphatase inhibitors (*5*). However, until encountering the starch phosphorylase enzyme, it was assumed that suppression of acid phosphatases promoted sucrose only by making possible a greater supply of ATP and phosphorylated hexose precursors.

Since the leaf-enzyme preparations have hydrolyzed every organic

phosphate thus far tested, we would assume that a phosphoprotein, say starch phosphorylase, would also be attacked by one of the acid phosphatases. In this way, any starch-phosphorylase reaction favorable to sucrose production, *i.e.*, one favoring phosphorolysis, with the production of glucose-1-phosphate, would automatically be retarded by any treatment stimulating the acid phosphatases. Conversely, treatments suppressing the acid phosphatases should retard the one which inactivates starch phosphorylase and thus increase sucrose-forming potential. Such phosphatase-sucrose relationships have consistently been observed during our investigations, and the possibility that a phosphorylase inactivator might be involved cannot now be ruled out.

The situation begins to become complex. We have already seen that the high-nitrate treatment, designed to promote starch phosphorylase, had greatly stimulated every phosphatase measured, and, in effect, our treatment must have stimulated the potential phosphorylase inactivator working within the same plant materials. Again, we must consider what happens within the plant tissues once this hypothetical inactivation has taken place. From our knowledge of animal phosphorylases, we understand that the enzyme would have to be reactivated by a specific kinase in conjunction with either ATP or AMP:

$$\text{Inactive phosphorylase} + \text{ATP or AMP} \xrightarrow[Mg^{2+} \text{ or } Mn^{2+}]{\text{Kinase}} \text{Active phosphorylase} + \text{ADP or Adenylic acid}$$

We also know that in sugarcane the same treatments which would stimulate the inactivating phosphatase also stimulate systems hydrolyzing ATP and ADP (table 2, Uba main effects; table 3, high nitrate in M.336). The same treatments promoting phosphorylase inactivation thus would destroy simultaneously the means by which phosphorylase could have been reactivated. This may be one of the reasons that added ATP has been found essential for maximum sucrose formation under "controlled" conditions (*4,12,24*).

INHIBITORS OF STARCH PHOSPHORYLASE IN CANE

In addition to the possible phosphatase-adenylic acid relationships discussed above, several additional factors need to be considered as possible inhibitors of the sugarcane enzyme. β-glycerophosphate, for example, is reported to be a noncompetitive inhibitor of muscle phosphorylase (*13*, p. 441). During our own investigations of sugarcane enzymes, we have employed β-glycerophosphate only as a representative substrate for the acid phosphatases, and we have no evidence that this compound actually

exists in sugarcane. However, because of the fact that an organic phosphate is at all able to inhibit one of the phosphorylases, consideration should be given to the possibility of other organic phosphates having the same property, with respect to other phosphorylases. Such an inhibitor in cane would undoubtedly come under attack by strong phosphatase action.

To summarize the phosphatases, we have seen that these enzymes would potentially serve as direct phosphoprotein inhibitors, that they might destroy components of the adenylic acid system needed to reactivate phosphoproteins, and that they might hydrolyze organic phosphates serving as noncompetitive inhibitors of phosphorylase. The potential role of this highly active group of enzymes is therefore considerably extended.

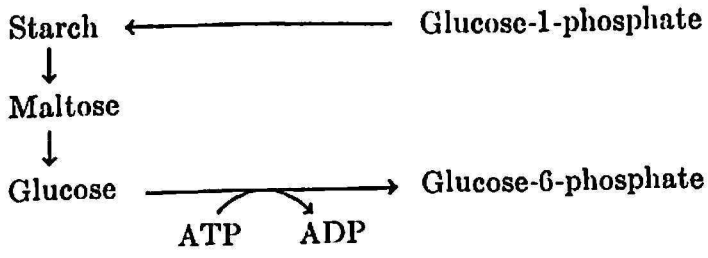
Glucose is a competitive inhibitor of the phosphorylase of muscle (*11*) and potato (*16*). By substituting for glucose-1-phosphate, glucose should theoretically block both phosphorolysis and the formation of polysaccharide. If such were the case with sugarcane, we would expect high-glucose levels to be associated both with low phosphorylase activity and low-sucrose levels. During the present investigations, we did in fact encounter relatively high glucose associated with relatively low sucrose in the leaves of Uba, and in the meristems of both Uba and M.336 (table 1). We also found relatively suppressed phosphorylase associated with the high glucose, as anticipated, in leaves of both Uba and M.336.

This same glucose-enzyme-sucrose relationship was found in meristems of Uba. It is interesting to note that the only samples where high glucose was not found associated with low sucrose (high-nitrate values for M.336 meristems, table 1), were likewise the only high-glucose samples where phosphorylase was apparently stimulated rather than suppressed. The confounding effect of high nitrate here might be regarded as a typical drawback of the factorial design, yet it indicates that our high-nitrate treatment, designed to stimulate starch phosphorylase through increased acidity, was apparently able to do so in the face of high-glucose values which in themselves were working to inhibit the same enzyme. High nitrate was not able to do so in Uba. Again, the relative preponderance of glucose in meristem rather than leaf tissues (table 1), may help account for the high-nitrate stimulation of starch phosphorylase in leaves rather than meristems.

Another point must be brought forward regarding the source of the potential glucose inhibitor. In this and a previous study (*2*) we found high amylase and high-starch phosphorylase operating, so to speak, hand-in-hand. One might logically expect that greater starch-forming potential would create a need for a starch-hydrolyzing system, and, in fact, this need by sugarcane to destroy starch was one of our primary conclusions of the previous work (*2*). Yet amylase represents the key hydrolytic system by

which large quantities of free glucose would be made available from starch. Therefore, a strong amylase system appearing simultaneously with high-starch phosphorylase would seem to provide a means for inhibiting the phosphorylase by free glucose, unless some other mechanism was present to prevent glucose from accumulating in the free form. The enzyme hexokinase was apparently serving such a purpose, for we find that system increasing its activity among the same treatments which stimulated amylase and starch phosphorylase, and decreasing its capacity where amylase and starch phosphorylase were suppressed (table 3, items 8, 9, and 12; and table 2, items 8, 9, 12, respectively).

Thus, the enzymes of the sequence,



seem to work together for the better utilization of glucose, as discussed in a previous paper (*2*), and for their own benefit as well.

ACTIVATION OF SUGARCANE STARCH PHOSPHORYLASE

As pointed out previously (*2*), the starch phosphorylase of cane preparations requires traces of polysaccharide in order to induce a strong reaction. The phosphorylases of both plant and animal tissues require a polysaccharide priming agent, which may be regarded as a true activator. The polysaccharide may be starch, glycogen, or dextrin. The primer takes a direct role in the reaction by providing a series of nonreducing ends of branched polysaccharide, upon which the enzyme catalyzes the addition of glucose-1-phosphate residues. Direct condensation of glucose-1-phosphate units proceeds very slowly in the absence of primer (*9*, p. 164). Straight chain polysaccharides, such as the amylose of starch, are ineffective, while branched-chain polysaccharides, such as amylopectin or glycogen, are excellent activators. The activating efficiency of a polysaccharide is therefore a function of the number of nonaldehydic terminal glucose units (*13*, p. 442).

We are fairly certain that at least two enzymes are involved in the formation of starch by cane preparations, or more accurately, in the formation of the polysaccharide which to this point we have termed "starch" for simplicity. The synthetic action of starch phosphorylase is confined to the formation of α, 1-4 glucosidic linkages, and consequently only straight-chain polymers are produced by this reaction (*13*, p. 444; *6*, p. 61). Never-

theless, it is well known that both starch and glycogen are composed of highly branched, long-chain polymers, consisting of both 1-4 and 1-6 glucosidic linkages. A hypothetical model, illustrating the straight-chain and branching relationships of glucose residues, is presented in figure 4.

It is generally accepted that the straight-chain polysaccharide, or "amylose", plus the highly branched polysaccharide, or "amylopectin", make up the two major components of starch (*6*, p. 49). An important question now arises: If starch phosphorylase can catalyze only the formation of straight-chain amylose, how are the 1-6 glucosidic bonds

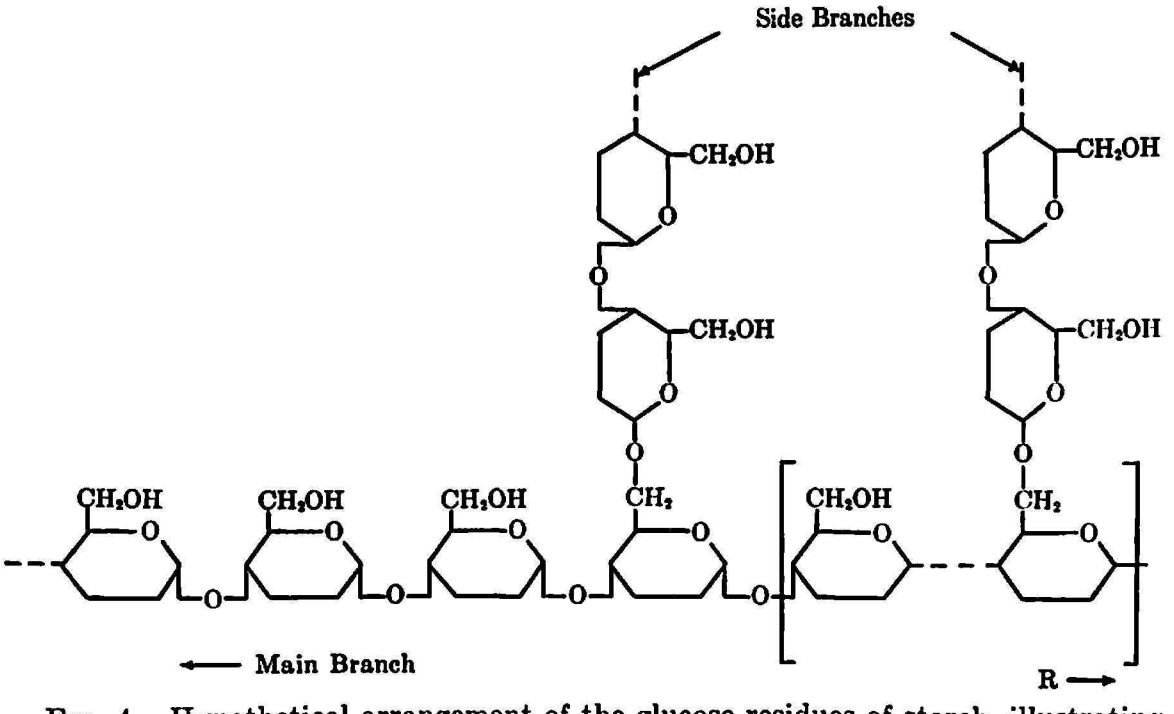


FIG. 4.—Hypothetical arrangement of the glucose residues of starch, illustrating the straight-chain, α, 1-4 linkages of amylose, and the 1-6 linkages which give rise to the branching structure of amylopectin.

of amylopectin (and glycogen) synthesized? From potato extracts a branching enzyme has been obtained, which, acting in conjunction with starch phosphorylase, forms a branched polysaccharide of the amylopectin type (*14,18*). This catalyst has been named the "Q enzyme". The Q enzyme apparently acts as a transglucosidase by transferring relatively short portions of a long, 1-4 linked amylose chain to the number 6 hydroxyl groups of glucose units within the chain. In effect, the 1-4 linked amylose chain is shortened and extensive branching is brought about through 1-6 linkages.

The straight-chain amylose of both starch and glycogen gives a deep blue color with iodine. However, the "starch" produced by the sugarcane starch phosphorylase gives a blue-violet color with iodine. According to Meyer (*17*), this blue-violet color is typical for branched polysaccharides. It has

been shown that, as amylose changes from the straight chain to an increasingly branched composition, the color of the iodine complex passes from blue to blue-violet, to red, and finally to brown. For this reason we strongly suspect that a catalyst synonymous with the Q enzyme of potato is active in sugarcane.

The presence of Q enzyme in sugarcane would in no way change our contention that starch formation and degradation is of paramount importance to the sucrose-producing capacity of cane. Yet, in future studies, at least one more enzyme would need attention as we try to improve the glucose-1-phosphate status through manipulation of starch phosphorylase. The Q enzyme would assume an importance equally significant as that already shown for amylase and starch phosphorylase.

SUMMARY AND CONCLUSIONS

In order to test the hypothesis that starch formation and metabolism is of importance to the sucrose-forming potential of cane, a series of 4-week-old plants was subjected to treatments designed to cause wide fluctuations in the enzyme starch phosphorylase. Treatments included high and low nitrate to vary acidity, high and low phosphate to vary the ratio of H_3PO_4 to glucose-1-phosphate, and two varieties (Uba and M.336) with distinct reputations for producing starch and sucrose, respectively. Treatments were supplied in $2 \times 2 \times 2$ factorial combination.

Sugar and enzyme assays were conducted with leaf and meristem samples harvested after 8 weeks of treatment. Sugars included total ketose, total reducing sugars, sucrose, fructose, and glucose. Enzymes included sucrose phosphorylase, starch phosphorylase, invertase, amylase, hexokinase, peroxidase, polyphenol oxidase, and a series of acid phosphatases hydrolyzing β-glycerophosphate, glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, 3-phosphoglyceric acid, ATP, and ADP. The following results were obtained:

1. A broad range of sucrose and enzyme levels was encountered between the Uba and M.336 varieties. Sugar variations appeared primarily in meristematic tissues, while enzyme differences were found in both leaves and meristems.

2. Overall enzyme activity was inversely related to the sucrose content of the meristem, with enzymes of M.336 active at about 60 percent of Uba values. Nitrate and phosphate effects were less pronounced.

3. Enzymes most suppressed by the M.336 treatment, as compared to Uba, were invertase, starch phosphorylase, amylase, hexokinase, and the phosphatases attacking ATP and ADP. Sucrose phosphorylase was generally constant for all treatments, indicating that sucrose variations were due to reactions less closely related to sucrose synthesis.

4. Invertase, starch phosphorylase, and the acid phosphatases appeared to be the systems most responsible for sucrose differences between Uba and M.336. It was suggested that starch phosphorylase was favoring polysaccharide formation in Uba, and phosphorolysis in M.336. The greater glucose-1-phosphate supply thus made available in M.336 was accompanied by a suppression of both glucose-1-phosphatase and invertase.

5. Nitrate, as anticipated, suppressed sucrose at the high level and promoted sucrose at the low level, in the Uba variety. But just the opposite effects were recorded in M.336. Nitrate also greatly affected the enzyme starch phosphorylase, in the planned manner, but the enzyme effects appeared in leaf tissues rather than in the meristematic areas where the sugar differences were most pronounced. The possibility of sugar translocation and the localization of certain enzyme reactions is discussed in detail.

6. Phosphate variables had but little effect on sucrose level, with the exception that high phosphate combined with high nitrate brought about the highest sucrose values recorded in M.336, while the same treatment in Uba yielded the lowest sucrose values of the study. The possible roles that organic phosphate and the acid phosphatases might serve as phosphorylase inhibitors are discussed in detail.

7. Low starch-phosphorylase activity was found to be associated with high glucose levels. Since glucose is known to be an inhibitor of muscle phosphorylase, it is possible that a similar glucose-enzyme relationship exists in sugarcane. Hexokinase and amylase appeared to fluctuate in general unison during this study, with the result that excess glucose liberated from polysaccharide should have been phosphorylated and made available for other functions than phosphorylase inhibitor.

8. Evidence suggests that a second enzyme, synonymous with "Q enzyme" of potato, is associated with starch phosphorylase of sugarcane. The enzyme would serve as an essential branching factor and thereby assume considerable importance in starch-sucrose relationships.

RESUMEN Y CONCLUSIONES

A fin de probar la hipótesis de que la formación del amidón y el metabolismo son importantes en la formación potencial de la sacarosa en la caña, se aplicaron tratamientos a una serie de plantas de 4 semanas de edad con el propósito de causar amplias fluctuaciones en la fosforilasa de almidón. Los tratamientos incluían cantidades altas y bajas de nitrato para variar la acidez y altas y bajas de fosfato para variar la proporción de H_3PO_4 a glucosa fosfatada-1, y dos variedades de caña de azúcar (Uba y M.336), las cuales se distinguen por su capacidad para producir almidón y sacarosa, respectivamente. Los tratamientos se aplicaron en una combinación factorial de $2 \times 2 \times 2$.

Se analizaron para azúcar y enzimas muestras de hojas y tejidos del meristemo, cosechadas 8 semanas después de los tratamientos. Los azúcares incluían la quetosa total, todos los azúcares reductores, sacarosa, fructosa y glucosa. Las enzimas incluían la fosforilasa de sacarosa, fosforilasa de almidón, invertasa, amilasa, hexoquinasa, peroxidasa, oxidasa de polifenol, y una serie de fosfatasas ácidas hidrolizadoras de glicerofosfato-β, fosfato glucosado-1, fosfato glucosado-6, fosfato fructosado-6, ácido fosfoglicérico-3, ATP y ADP. Se obtuvieron los siguientes resultados:

1. Se encontró una amplia sucesión de niveles de sacarosa y enzimas entre las variedades Uba y M.336. Las variaciones de azúcar aparecieron, principalmente, en los tejidos meristemáticos, mientras que las diferencias enzimáticas se encontraron en ambos: hojas y meristemos.

2. Toda la actividad enzimática estaba relacionada, inversamente, al contenido de sacarosa del meristemo, y en el caso de las enzimas de la M.336 la actividad alcanzó alrededor del 60 por ciento de los valores de la Uba. Los efectos del nitrato y del fosfato fueron menos evidentes.

3. Las enzimas que más se suprimieron mediante el tratamiento de la M.336, comparado con el que se le dió a la Uba, fueron la invertasa, fosforilasa de almidón, amilasa, hexoquinasa y las fosfatasas que actuaron sobre la ATP y la ADP. La fosforilasa de sacarosa fue generalmente constante en todos los tratamientos, indicando que las variaciones de la sacarosa se debieron a reacciones menos estrechamente relacionadas a la síntesis de la sacarosa.

4. La invertasa, la fosforilasa de almidón y las fosfatasas ácidas aparentemente fueron los sistemas más responsables de las diferencias en sacarosa entre la Uba y la M.336. Esto indujo a creer que la fosforilasa de almidón favorecía la formación de polisacaridas en la Uba, y la fosforólisis en la M.336. El abasto mayor de fosfato glucosado-1 que, en consecuencia hízose disponible en la M.336, se acompañó de una supresión de glucosa fosfatasa-1 e invertasa.

5. El nitrato, como se anticipó, suprimió la sacarosa a un alto nivel y la estimuló a un bajo nivel, en la variedad Uba. Pero, por otro lado, efectos contrarios se produjeron en la M.336. El nitrato afectó grandemente la enzima fosforilasa de almidón, según se esperaba; pero los efectos enzimáticos aparecieron en los tejidos de la hoja, más bien que en las áreas meristemáticas donde las diferencias en el contenido de azúcar fueron más pronunciadas. También se discute en detalle la posibilidad de la translocación de azúcar y de la localización de ciertas reacciones enzimáticas.

6. Las variables de fosfato tuvieron muy poco efecto sobre el nivel de sacarosa, excepto que cuando se combinaron altas aplicaciones de fosfato y nitrato hubo la más alta producción de sacarosa en la M.336; mientras que el mismo tratamiento en la Uba rindió los valores más bajos de sacarosa

en este estudio. Se discute en detalle la posible función que desempeñe el fosfato orgánico y las fosfatasas ácidas como inhibidores de la fosforilasa.

7. Se encontró que la baja actividad de la fosforilasa de almidón está asociada con altos niveles de glucosa. Y, toda vez que la glucosa es un conocido inhibidor de la fosforilasa que actúa sobre el sistema muscular, es posible que en la caña de azúcar se produzca una relación similar entre la glucosa y la enzima. Se encontró, durante el estudio, que la hexoquinasa y la amilasa parecieron fluctuar conjuntamente, con el resultado de que el exceso de glucosa liberada de las polisacaridas debió haber sido "fosforilado" y haber estado disponible para otras funciones, además de la de inhibidor de la fosforilasa.

8. La evidencia sugiere la presencia de una segunda enzima, sinónima de la "enzima Q" de la papa, la cual está asociada con la fosforilasa de almidón de la caña de azúcar. La enzima serviría como un factor ramificador esencial, por lo que asumiría una considerable importancia en las relaciones entre el almidón y la sacarosa.

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. ——, Physiological studies of enzymes catalyzing the synthesis and hydrolysis of sucrose, starch, and phosphorylated hexose in sugarcane, *J. Agr. Univ. P.R. 49*(1): 60–75, 1965.
3. ——, 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.
4. ——, The biosynthesis of sucrose in sugarcane, *J. Agr. Univ. P.R. 48* (4): 265–83, 1964.
5. ——, Induction of varying sugar levels in leaves of immature sugarcane by use of acid phosphatase inhibitors, *J. Agr. Univ. P.R. 49* (1): 35–59, 1965.
6. Bonner, J., Plant Biochemistry, Academic Press Inc., New York 10, N.Y., 1950.
7. Cardini, C. E., Leloir, L. F., and Chiriboga, J., The biosynthesis of sucrose, *J. Biol. Chem. 214:* 149–55, 1955.
8. Clark, H., Effect of ammonium and nitrate nitrogen on the composition of the tomato plant, *Plant Physiol. 11:* 5–24, 1936.
9. Conn, E. E., and Stumpf, P. K., Outlines of Biochemistry, John Wiley & Sons, Inc., New York, N.Y., 1963.
10. Cori, C. F., Colowick, S. P., and Cori, G. T., The isolation and synthesis of glucose-1-phosphoric acid, *J. Biol. Chem. 121:* 465–77, 1937.
11. Cori, G. T., and Cori, C. F., The kinetics of the enzymatic synthesis of glycogen from glucose-1-phosphate, *J. Biol. Chem. 135:* 733–56, 1940.
12. Dugger, W. M., and Humphreys, T. E., Influence of boron on enzymatic reactions associated with biosynthesis of sucrose, *Plant Physiol. 36:* 523–30, 1960.
13. Fruton, J. S., and Simmonds, Sofia, General Biochemistry, John Wiley & Sons, New York, N.Y., 1959.
14. Gilbert, G. A., and Patrick, A. D., Enzymes of the potato concerned in the syn-

thesis of starch, I, The separation and crystallization of Q-enzyme, *Biochem. J. 51:* 181–6, 1952.

15. Green, A. A., and Cori, G. T., Crystalline muscle phosphorylase, I, Preparation, properties, and molecular weight, *J. Biol. Chem. 151:* 21–9, 1943.
16. Hanes, C. S., Reversible formation of starch from glucose-1-phosphate catalyzed by potato phosphorylase, *Proc. Roy. Soc. London B 129:* 174–208, 1940.
17. Meyer, K., The chemistry of glycogen, *Advances in Enzymol. 3:* 109–35, 1943.
18. Peat, S., The biological transformations of starch, *Advances in Enzymol. 2:* 339–75, 1951.
19. Pucher, G. W., Leavenworth, C. S., Ginter, W., and Vickery, H. B., Studies in the metabolism of Crassulacean plants; the effect upon the composition of *Bryophyllum calycinum* of the form in which nitrogen is supplied, *Plant Physiol. 22:* 205–27, 1947.
20. Rall, T. W., Sutherland, E. W., and Wosilait, W. D., The relationship of epinephrine and glucagon to liver phosphorylase, III, Reactivation of liver phosphorylase in slices and in extracts, *J. Biol. Chem. 218:* 483–95, 1956.
21. Roe, J. H., A colorimetric method for the determination of fructose in blood and urine, *J. Biol. Chem. 107:* 15–22, 1934.
22. Sumner, J. B., Dinitrosalicylic acid: A reagent for the estimation of sugar in normal and diabetic urine, *J. Biol. Chem. 47:* 5–9, 1921.
23. Sutherland, E. W., Cori, C. F., Haynes, R., and Olsen, N. S., Purification of hyperglycemic-glycogenolytic factor from insulin and from gastric mucosa, *J. Biol. Chem. 180:* 825–37, 1949.
24. Turner, J. F., Synthesis of sucrose by a plant enzyme extract, *Nature* London *172:* 1149–50, 1953.
25. Van Dillewijn, C., Botany of Sugarcane, The Chronica Botanica Co., Waltham, Mass., p. 315, 1952.
26. Vickery, H. B., Pucher, G. W., Wakeman, A. J., and Leavenworth, C. S., Chemical investigations of the tobacco plant, VIII, The Effect upon the Composition of the Tobacco Plant of the Form in Which Nitrogen is Supplied, Conn. Agr. Expt. Sta. B. 442, 65–119, 1940.
27. Wadleigh, C. H., and Shive, J. W., Organic acid content of corn plants as influenced by pH of substrate and form of nitrogen supplied, *Amer. J. Bot. 26:* 244–8, 1939.
28. Weller, D. M., Accumulations of starch in the stalks of Natal Uba cane grown on acid soil, *Hawaii Planters Rec. 33:* 294–9, 1929.
29. Wood, G. G., Some Factors Influencing Starch in Sugarcane, Proc. S. African Sugar Technol. Assn. pp. 123–35, April 1962.