rate of elongation of immature internodes were linearly related, both increasing with increasing temperature provided the plant had adequate water. Changes in the total sugar content of mature tissue were related to the neutral invertase activity of the tissue. The neutral invertase activity and sugar content of mature internodes increased as the temperature was decreased. The results are discussed in relation to the concept that invertases of sugar cane storage tissue contribute to the partitioning of available carbohydrate from photosynthesis between the processes of sugar storage and growth.

Acknowledgment

The technical assistance of Mr. A. A. Cillekens is gratefully acknowledged.

Literature Cited

1. GLASZIOU, K. T. 1962. Accumulation & transformation of sugars in sugar cane stalks. Mechanism of inversion of sucrose in the inner space. Nature 193: 1100.
2. HATCH, M. D., J. A. SACHER, & K. T. GLASZIOU. 1963. The sugar accumulation cycle in sugar cane. I. Studies on the enzymes of the cycle. Plant Physiol. 38: 338–43.
3. HOFFMAN, W. S. 1937. A rapid photometric method for the determination of glucose in blood & urine. J. Biol. Chem. 120: 51–5.
4. SACHER, J. A., M. D. HATCH, & K. T. GLASZIOU. 1963. The sugar accumulation cycle in sugar cane. III. Physical & metabolic aspects of the cycle in immature storage tissue. Plant Physiol. 38: 348–54.
5. WENT, F. W. 1961. Temperature. In: Encyclopedia of Plant Physiology. W. Ruhland, ed. Springer-Verlag. vol. XVI: 1–22.

Sugar Accumulation Cycle in Sugar Cane. III. Physical & Metabolic Aspects of Cycle in Immature Storage Tissues[1]

J. A. Sacher[2], M. D. Hatch, & K. T. Glasziou

David North Plant Research Centre, Colonial Sugar Refining Co. Ltd., Indooroopilly, Queensland, Australia

Introduction

Sugar cane storage tissues accumulate sugar against a concentration gradient using energy provided by respiration (2). Kinetic and tracer studies show that tissue discs placed in sugar solutions have three distinct compartments (6, 7). These compartments are redefined with specific reference to sugar accumulation. The outer space which includes the cell walls is the zone of the tissue in rapid diffusion equilibrium with sugars and ions in the bathing solution. The metabolic compartment is a zone in which hexoses are phosphorylated and interconverted. Movement of anions including phosphorylated sugars between the bathing solution and this zone is restricted. It is also assumed to be the locus of synthesis of a sucrose derivative termed sucrose-X, cleavage of which provides the necessary energy for accumulation of sucrose against a gradient into the storage compartment. The storage compartment is the zone in which sucrose, glucose, and fructose are accumulated and which is bounded by a permeability barrier resistant to diffusion of sugars.

For tissue slice studies on sugar storage we use the following terms. Uptake describes the total amount of sugar removed from the medium and retained by the tissue after washing, irrespective of its form. That part of the total sugars taken up which appears as sugar in the storage compartment is defined as accumulation.

Sucrose is virtually the sole sugar transferred from the metabolic to the storage compartment. Reducing sugars in the storage compartment are derived from hydrolysis of accumulated sucrose (7). All sugars diffuse slowly from the storage compartment, reducing sugars diffusing more rapidly than sucrose (6). Immature storage tissue contains an invertase which is optimally active between pH 5.0 and 5.5 (8, 11). The enzyme is absent from more mature tissue which contains higher total sugar but

[1] Received Sept. 28, 1962.
[2] Supported in part by Research Grant (G-9894) from the National Science Foundation. Permanent address: Los Angeles State College, Los Angeles 32, Cal.

little reducing sugar. It is thought that this enzyme could be responsible for inversion of sucrose in the storage compartment.

Studies on sucrose uptake have indicated that two parallel pathways may be involved, one possibility being direct conversion of sucrose to sucrose-X (7). However, in Canna leaves Hassid and co-workers (9) concluded that sucrose is inverted in the intercellular spaces prior to accumulation. The work to be reported was undertaken to clarify some aspects of sucrose uptake and to obtain further information on the role of invertase in sugar storage.

Methods & Materials

Sugar Accumulation. A commercial variety of sugar cane (NCO310) was used. Tissue discs (5.0 × 0.5 mm) were prepared from the basal portion of immature internodes and washed for 1 hour in running tap water. The discs were then placed into radioactive sugar solutions in 10 ml conical flasks and incubated for 4 hours at 30° in a shaker, followed by washing for 1 hour in running tap water to remove sugars from the outer space (6, 7). Three volumes of hot absolute ethanol were added to the blotted discs and the mixture was shaken for 16 hours to extract the sugars.

Radioactive Assay. Radioactive sugars were obtained from the Radiochemical Center, Amersham. Aliquots of ethanol extracts were co-chromatographed with non-radioactive markers (glucose, fructose, & sucrose) on Whatman No. 1 paper using descending chromatography with ethyl acetate: pyridine: H_2O (8:2:1) as the developing solvent. Radioactive sugars were assayed directly on the paper, after detection with p-anisidine phosphate, by use of a thin end window Geiger-Müller tube. Alternatively the chromatograms were scanned with a recording strip-counter and the radioactivity in different sugars determined by measuring the area of peaks with a planimeter. The distribution of C^{14} in sucrose was determined by eluting the sucrose and re-applying it to Whatman No. 1 paper. The baseline spot of sucrose was treated with analytical invertase, dried, then chromatographed and radioactivity in glucose and fructose assayed as described above.

Invertase Assay. Washed tissue discs were ground in a mortar and the juice expressed through fine muslin and dialyzed to remove sugars. Juice and cell residue were assayed separately in reaction mixtures containing 1 % sucrose-U-C^{14} at pH 5.5 and 30°. Hydrolysis was generally measured over a period of 4 hours or less. Toluene, 0.05 ml was always added. At hourly intervals 5 μl aliquots of reaction mixtures were applied to Whatman No. 1 paper and chromatographed for subsequent radioactive assay as described above. Chromatographed samples contained approximately 3000 cpm.

Outer space invertase was determined by incubating washed discs in a 1 % sucrose-U-C^{14} solution with a buffer containing equimolar amounts of citrate and phosphate titrated to the desired pH. Methods used for calculating outer space invertase are described and discussed in the text.

Preparation of Asymmetrically-labeled Sucrose. Sucrose labeled in the fructosyl moiety was synthesized enzymically using a UDP-glucose-fructose transglucosylase preparation from sugar cane. Fructose-U-C^{14}, 27.4 μmoles and UDP-glucose, 30 μmoles, were incubated with the enzyme in 0.05 M tris-HCl buffer (pH 8.3). The mixture was boiled to stop the reaction and the precipitated protein removed by centrifugation. The labeled sucrose was purified by chromatography. Treatment of the purified sucrose with analytical invertase showed that only the fructose moiety of sucrose was labeled. The preparation was free of other labeled compounds.

Results

Cyclical Nature of Sugar Accumulation Process. We first determined whether sugar moving from the storage space may be returned to storage. Tissue discs were supplied with glucose-U-C^{14} for 10 hours, then extensively washed to remove outer space sugar. One sample of discs was then suspended in a humid atmosphere, thus forming a closed system. Discs of another sample were crushed with a glass rod. The movement of label in sugars of intact and crushed discs was then followed (fig 1). In crushed discs

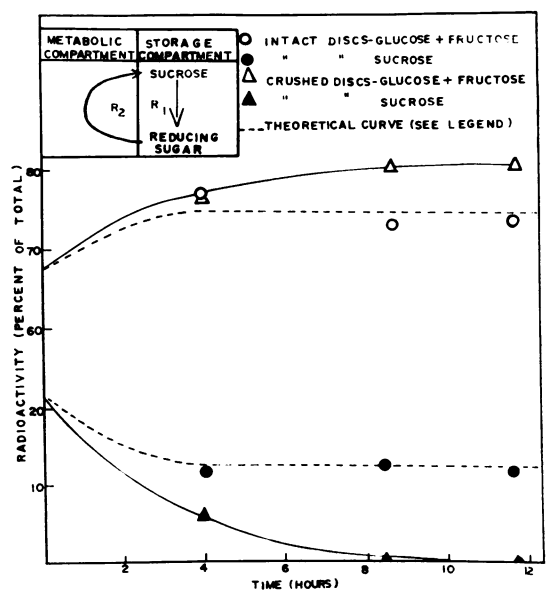

FIG. 1. Movement of radioactivity between sucrose and reducing sugars in intact and crushed storage tissue discs. Discs previously immersed in glucose-U-C^{14} were washed to remove outer space sugars, then 20 discs were either suspended in a stoppered tub or homogenized. Toluene was added to the crushed discs. At intervals replicate samples were used to determine radioactivity in sugars (see Methods and Materials section). The theoretical curve represented by the broken line was derived from the model system in the inset.

all activity in sucrose disappeared and was accompanied by a concomitant rise in reducing sugars. In intact discs, the activity in sucrose declined to a steady level while that in reducing sugars increased to a steady level. In contrast to this result the radioactivity moved out of sucrose and reducing sugars with intact discs in an open system containing unlabeled glucose in the medium (7).

A simplified model system illustrating the cyclical process is depicted in the inset of figure 1. The assumption is made that virtually all of the label moving from sucrose to the reducing sugars in the storage space passes to the metabolic compartment and thence back to the storage space as sucrose (in fact, 10 % of the total radioactivity of compounds soluble in 70 % ethanol was lost during the time course of the experiment).

The movement of label into and from the sucrose pool is described by the equation

$$\frac{dS^*}{dt} = -\frac{R_1 S^*}{S} + \frac{R_2 H^*}{H} \qquad \text{I}$$

where: S* and H* are radioactivities of sucrose and reducing sugars; S and H are the steady state sucrose and reducing sugar contents of the storage space; R_1 and R_2 are the transfer rates from sucrose to reducing sugar and vice versa, the transfer rate being the product of the velocity constant for the reaction and the concentration of substance in the compartment (16).

R_1 may be estimated from the loss of radioactivity from sucrose in crushed discs. Invertase activity is responsible for sucrose inversion in the storage space (7, 8). For crushed discs, the second term of equation I may be neglected. Integration and re-arrangement gives

$$R_1 = S \times t \times 2.3 \log \frac{So^*}{S^*} \qquad \text{II}$$

An arbitrary value of 100 units is assigned for the sucrose content. From figure 1 So* is 21 % and S* is 14 % after 1 hour. Substitution into (II) gives the transfer rate as 40 units per hour. Changes in the radioactivity of the reducing sugar pool are given by

$$\frac{dH^*}{dt} = \frac{R_1 S^*}{S} - \frac{R_2 H^*}{H}$$

At a steady rate dH*/dt is zero and R_1 equals R_2. Hence

$$H = \frac{H^*}{S^*} \times S \qquad \text{III}$$

From the experimental data S* is 12 and H* 72 % of total activity at steady state conditions so that H is 600 units. The theoretical curves derived from these values for the model system are plotted as broken lines in figure 1, and agree with the experimental values.

Symmetry of Labeling of Sucrose Accumulated into Storage Compartment. Sucrose accumulated by immature storage tissues, which contain endogenous glucose and fructose, was equally labeled in the hexose moieties when glucose-U-C^{14} was supplied in the medium (6). This result was confirmed and extended to the situation with fructose-U-C^{14} as the sugar in the external medium (table I). When asymmetrically labeled sucrose was supplied considerable randomization of the label occurred. Furthermore the sucrose accumulated from a mixture of labeled sucrose-U-C^{14} and unlabeled fructose was strongly asymmetric. The ratio of label in glucose to fructose moieties of 3.25 obtained in the latter instance (table I), shows that a minimum of 70 % of the sucrose-U-C^{14} entering the tissue was hydrolyzed during movement to the storage compartment. We emphasize that the distribution of label in hexose moieties of accumulated sucrose was determined after all outer space sugar had been removed by exhaustive washing.

Table I

Radioactivity in Glucose & Fructose Moieties of Sucrose Accumulated from Labeled & Unlabeled Sugars

Incubation media*		Ratio C^{14} in glucosyl to fructosyl moieties
Radioactive sugar	Unlabeled sugar	
0.055 M fructose-U-C^{14}	...	0.97
0.03 M sucrose-U-C^{14}	...	1.04
0.03 M sucrose-U-C^{14}	0.055 M fructose	3.25
0.015 M fructosyl-U-C^{14} sucrose	...	0.33**

* Immature storage tissue discs (0.3 g fr wt) were incubated for 4 hours in 1 ml of unbuffered solution containing 1.6×10^6 cpm of either fructose-U-C^{14} or sucrose-U-C^{14} or 0.6×10^6 cpm of fructosyl-U-C^{14} sucrose (19 % counting efficiency). A sample of accumulated sucrose containing at least 2000 cpm was used to determine the distribution of label in the hexose moieties. Analytical procedures are described in the Methods and Materials section.

** Initial ratio less than 0.001.

Distribution of C^{14} in Sucrose Accumulation from a Solution of Glucose-1-C^{14}. Discs were incubated in a medium of glucose-1-C^{14} for 4 hours, the accumulated sucrose isolated and hydrolyzed with analytical grade yeast invertase. Following chromatographic separation, it was shown that the glucosyl and fructosyl moieties were equally labeled. Osazone derivatives of the hexoses were treated with periodic acid as described by Aronoff (1). The mesaxoldehyde osazone (containing carbon atoms 1, 2, & 3 of the hexoses) was isolated and found to contain 95 % of the original radioactivity of the hexoses. Hence it may be concluded that reversible breakdown of glucose to triose phosphate did not proceed to a significant extent during the transformation of sugars leading to sucrose accumulation.

Distribution of Invertase. Two invertases can be extracted from sugar cane, one with a pH optimum between 5.5 and 5.0 and the other 7.0 (11). The enzyme with a low pH optimum (acid invertase) is found in large amounts in actively-growing immature tissue. The pH 7.0 enzyme (neutral invertase) is found only in mature tissue.

Invertase activity in the outer space of immature tissue discs was demonstrated qualitatively by the appearance of labeled glucose and fructose in a medium which contained sucrose-U-C^{14}. The amount of hexose in the bathing solution would depend upon the balance between sucrose hydrolysis and the uptake of hexose by the tissue, and hence could not be used as a measure of outer space invertase. After uptake of sucrose-U-C^{14} much larger quantities of labeled fructose were present in the medium than labeled glucose. This was consistent with a substantial uptake of hexoses formed from sucrose-U-C^{14} with glucose being taken up much more rapidly than fructose. Separate experiments showed that with 0.1 % solutions glucose uptake was three times faster than fructose.

By assuming that the amount of sucrose taken up by the tissue without prior inversion was negligible, loss of sucrose from the medium could be used as a measure of outer space invertase. Calculations of the amount of hexose formed from sucrose, and taken up by the tissue agreed closely with measurements of the total uptake of radioactivity by the tissue. The calculation was based on the observed differences in the rate of glucose and fructose uptake (separate experiments) and the amounts of glucose and fructose remaining in the medium after the period of uptake. Labeled hexose in the medium plus radioactivity taken up into the tissue, used as a measure of the products of invertase action, provided an alternative method for calculating outer space invertase. Determinations by the two methods were in agreement.

Table II shows the distribution of invertase activity in tissue taken from basal portion of immature internodes which includes the region of the intercalary meristem. The tissue was washed for 1 hour prior to assay for outer space invertase. From 18 to 69 % of the total invertase was present in the outer space in different batches of this tissue. The activity associated with the cell residue, which would include cell wall material, accounted for only a small proportion of the outer space invertase activity. Microscopic examination showed that the 0.5 mm discs contained 14 to 20 cell layers, from which it may be calculated that cut cells did not contribute significantly to the result. To check on the contribution by cut cells to outer space invertase activity, assays were made on unwashed discs, and discs washed in running water. About 14 % of the activity of unwashed discs was lost after 30 minutes washing, and no further decline occurred when the washing period was extended to 120 minutes.

Table II

Distribution of Invertase in Discs of Immature Storage Tissue

Exp.	Invertase Activity*				
	Juice	Residue	Total	Outer space	Outer space (% total)
1	3950	262	4212	1557	37
2	2160	130	2290	860	38
3	3800	225	4025	708	18
4	3123	311	3434	2360	69

* μg sucrose hydrolyzed/g fresh wt/hr. Assay procedures are described in the Methods and Materials section. For the determination of outer space invertase activity tissue discs were supplied with sucrose-U-C^{14} as described in table I. The method used to calculate outer space invertase activity is described in the Results section.

Effect of pH on Outer Space Invertase Activity. Relationship of Outer Space Invertase to Sucrose Accumulation. The activity of outer space invertase varied with pH of the medium reaching an optimum value at approximately pH 5.0 (fig 2). There was no significant effect of pH between 5.5 and 7.8 on sugar accumulated from glucose. At low pH the accumulation from glucose was enhanced, possibly due to movement of buffer anions (citrate & phosphate) into the cytoplasm. The accumulation curve for sucrose at different pH values was very similar

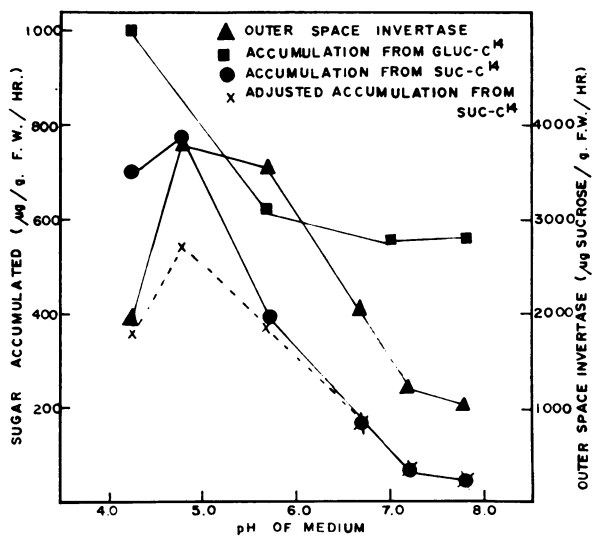

FIG. 2. Outer space invertase activity and sucrose and glucose accumulation as a function of pH. Each flask contained 15 discs (0.21 g) of immature storage tissue, either 1 % sucrose-U-C^{14} or 1 % glucose-U-C^{14}, and a citrate-phosphate buffer (0.05 M with respect to each anion) adjusted to the desired pH. The final liquid volume was 0.5 ml. The basis for the adjustment of the sucrose accumulation curve is described in the text.

Table III

Effects of Tris on Outer Space Invertase & Uptake of Sucrose & Glucose

Substrate for uptake	pH	Uptake* μg/g fr wt-hr		Inhibition of uptake %	Inhibition of outer space invertase %
		−tris	+tris		
Sucrose-C^{14}	5.7	630	505	21%	49%
"	7.2	286	130	55%	68%
"	7.8	206	54	74%	84%
Glucose-C^{14}	7.2	803	740	8%	...
"	7.8	910	770	14%	...

* Conditions and procedure used for uptake studies were as described in the Materials and Methods section. Uptake was determined from the counts extractable in 70% ethanol from washed tissue and includes stored sugars. The final concentration of tris was 0.025 M.

to that for outer space invertase. This correlation was even more apparent if a correction was applied based on the enhanced accumulation from glucose at low pH; an effect which is probably unrelated to the normal accumulation process. A similar relationship was found for sucrose uptake and outer space invertase. These results provide evidence that inversion in the outer space is an integral step in sucrose uptake, and show that this step may control the rate of uptake.

Hatch et al. (11) have shown that tris inhibits the activity of acid invertase extracted from sugar cane, the inhibition being greater at higher pH. The effects of tris on outer space invertase activity and uptake of sucrose or glucose by tissue discs is shown in table III. Marked inhibition of outer space invertase and sucrose uptake, but not glucose uptake, provides support for the concept that inversion of sucrose in the outer space is essential for sucrose uptake.

Discussion

A schematic representation of sugar transformations associated with movement of sugars between cellular compartments is shown in figure 3 which modifies and extends a scheme presented previously (7). The extensions are based on current studies of enzymes and sugar transformations which occur in cane (10, 11, 15). The only important modification is in the explanation offered for parallel pathways for sucrose uptake which is now given in terms of differential rates of phosphorylation of glucose and fructose rather than by direct conversion of sucrose to sucrose-X. The change is made because of our finding that the outer space acid invertase limits the rate of sucrose uptake in some batches of immature tissue. In other batches we find this enzyme is present in excess, and glucose and fructose accumulate in a medium which initially contained sucrose alone. This result, renders invalid an alternative interpretation of sugar uptake in immature storage tissue of cane which was in terms of a common, rate-limiting, carrier for sucrose, glucose, and fructose uptake (3).

Bieleski (2) has pointed out that most previous studies of sugar accumulation in plant tissues have failed to differentiate between diffusion into the free space (which includes cell walls) and metabolically-mediated accumulation against a concentration gradient. In the work reported by Putman and Hassid (13), it appears probable that the movement of radioactivity from glucose-C^{14} or fructose-C^{14} which had been infiltrated into Canna leaf discs, was movement from the free space into sucrose without any equilibration with the endogenous hexose pools. The radioactivity appearing in sucrose was found to be distributed equally between the two hexose moieties. We obtained qualitatively similar results when glucose-C^{14} or fructose-C^{14} were supplied alone in that sucrose accumulated into the storage compartment was equally labeled in both hexose moieties

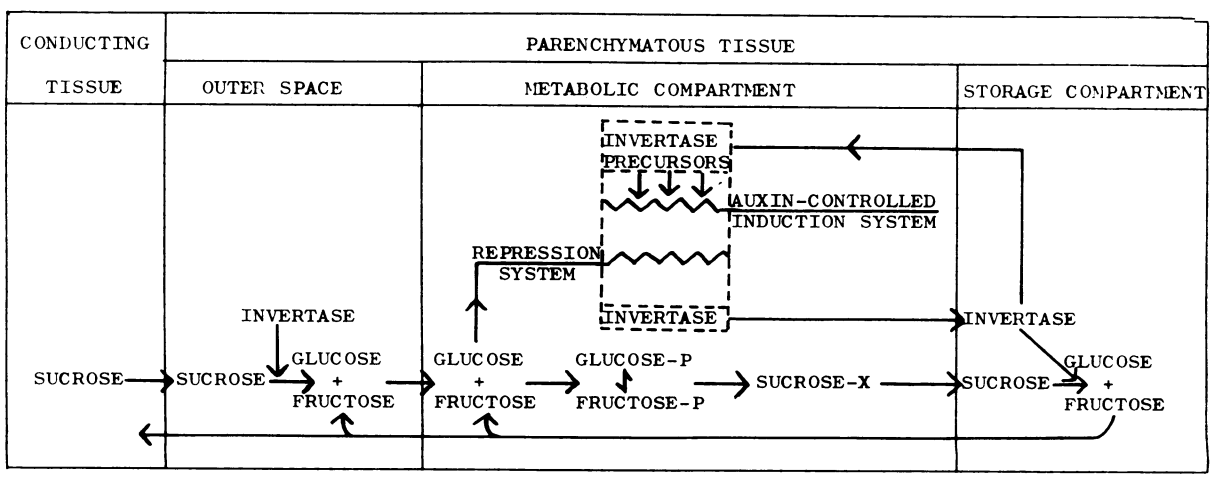

FIG. 3. Schematic representation of the sugar accumulation cycle in sugar cane storage tissue.

despite the presence of endogenous hexoses in the tissue. The asymmetry of labeling in accumulated sucrose, which occurred when we supplied mixtures of sucrose-U-C^{14} and unlabeled fructose, provides more conclusive evidence for the spatial separation of the metabolic and storage compartments of cane tissue than has been given previously (7).

A second feature which Canna leaf discs and immature cane storage tissues have in common is an outer space invertase. However, a major difference is apparent in the origin of the endogenous hexose pools which, in cane, have been shown to arise from sucrose accumulated into the storage compartment (6, 7, & this paper). For Canna leaf, no movement of label from accumulated sucrose into the endogenous hexoses was detected. Hence the sugar cycle described herein for cane tissue does not appear to operate in Canna leaf and, in fact, the origin of the hexoses in this photosynthetic tissue is unexplained.

There have been several reports of high invertase activities in rapidly growing plant tissues (12, 14, 17). We find an approximately linear relationship between the rate of elongation of immature internodes, and the invertase content per gram fresh weight of tissue (10). This invertase has optimum activity between pH 5.0 and 5.5 and is located in two separate compartments, the storage compartment and the outer space. We think the outer space enzyme is confined to the cell wall or cell surface, and suggest that its function is to control the flow of sucrose from the conducting tissue to the young growing cells. It can do this because sucrose must first be hydrolyzed then resynthesized during transfer through the metabolic compartment to the storage compartment. We suggest the role for the invertase in the storage compartment is concerned with the rate of return of sugar from storage which will be a function of the concentration of the enzyme if the vacuolar membrane is more permeable to hexoses than to sucrose. The feasibility of this proposition has been demonstrated by the construction of artificial membranes having such properties (5); also direct evidence was provided by the leakage studies reported earlier (6). Apparently a regulatory system for controlling the enzyme level is coupled to the amount of hexose present in the metabolic compartment since when glucose or fructose are supplied to tissue slices there is a rapid decline in the content of the inner space invertase (15). Increase in the level of inner space invertase occurs in response to auxin (15), nitrogen supply, and osmotic gradient across the cell (unpublished results). The response to the external conditions is fast; for example the half-time for loss of the enzyme is about 4.5 hours, and under special conditions a tenfold increase may occur in 6 hours. Rapid adjustments of the level of invertase in immature tissue occur in intact plants in response to temperature changes and water stress (10). These observations are such as could be anticipated for an enzyme fulfilling the key regulatory role we have postulated.

The acid invertase is not present in the mature tissue of cane varieties with a high capacity for sucrose storage, but is replaced by a neutral invertase with optimum activity of pH 7.0. As with the acid invertase, the level of neutral invertase is readily increased or decreased by changes of temperature or water stress (10). We suggest it has a similar function for directing movement of photosynthate to that which we propose for the outer space acid invertase of immature tissue.

The concept of a circulatory system in the cane plant was developed by Burr and co-workers on the basis of many experiments and field observations. Among these was their demonstration that radioactive carbon, fixed photosynthetically from CO_2 supplied to a single leaf, moves to all stalks in a stool of cane (4). Our work on intact cane stalks has shown that sugar may be returned from storage for local utilization (6) or may be translocated and used elsewhere (10). The scheme for the sugar accumulation cycle in immature internodes provides a framework of reference within which many observations at the sub-cellular, cellular, and whole plant levels may be related.

Summary

I. There are three distinct cell compartments through which sugars move when accumulated from the medium into storage tissue discs. The compartments, termed the outer space, the metabolic compartment and the storage compartment are characterized metabolically and by their behaviour with respect to diffusion of sugars and anions.

II. Sucrose is inverted in passing from the medium into the storage compartment where it reappears as sucrose. Inversion is mediated by an acid invertase in the outer space and is apparently an integral step in the accumulation of sucrose. Under certain conditions it may be rate limiting. The invertase in the outer space is only a part of the total acid invertase activity of the tissue. At least a part of the remaining activity is in the storage compartment.

III. A cyclic scheme in which sugars are moved to the storage compartment by an active process and lost from the compartment by diffusion is proposed. This system is termed the sugar accumulation cycle. Various interrelations between growth and sugar storage in sugar cane plants are discussed in relation to the cycle.

Literature Cited

1. ARONOFF, S. 1956. In: Techniques of Radiobiochemistry, Iowa College Press, pp 109–10.
2. BIELESKI, R. L. 1960. The physiology of sugar cane. III. Characteristics of sugar uptake in slices of mature & immature storage tissue. Australian J. Biol. Sci. 13: 203–20.

3. Bieleski, R. L. 1962. The physiology of sugar cane. V. Kinetics of sugar accumulation. Australian J. Biol. Sci. 15: 429–44.
4. Burr, G. O., T. Tanimoto, C. E. Hartt, A. Forbes, G. Sadaoka, F. M. Ashton, J. H. Payne, J. A. Silva, & G. E. Sloane. 1958. In: Nuclear Radiation in Food & Agriculture. W. R. Singleton, ed. D. Van Nostrand Co. Princeton, New Jersey. Chapter 4: 59–70.
5. Craig, L. C. & A. O. Pulley. 1962. Dialysis studies. IV. Preliminary experiments with sugars. Biochemistry 1: 89–94.
6. Glasziou, K. T. 1960. Accumulation & transformation of sugars in sugar cane stalks. Plant Physiol. 35: 895–901.
7. Glasziou, K. T. 1961. Accumulation & transformation of sugars in sugar cane. Origin of glucose & fructose in the inner space. Plant Physiol. 36: 175–79.
8. Glasziou, K. T. 1962. Accumulation & transformation of sugars in sugar cane stalks: Mechanism of inversion of sucrose in the inner space. Nature 193: 1100.
9. Hassid, W. Z. 1958. The synthesis & transformations of the oligosaccharides in plants, including their hydrolysis. In: Encyclopedia of Plant Physiology, W. Ruhland, ed. 6: 125–36. Springer-Verlag, Berlin.
10. Hatch, M. D. & K. T. Glasziou. 1963. The sugar accumulation cycle in sugar cane. II. Relationship of invertase activity to sugar content & growth rate in storage tissue of plants grown in controlled environments. Plant Physiol. 38: 344–48.
11. Hatch, M. D., J. A. Sacher, & K. T. Glasziou. 1963. The sugar accumulation cycle in sugar cane. I. Studies on the enzymes of the sugar cycle. Plant Physiol. 38: 338–43.
12. Hellebust, J. A. & D. F. Forward. 1962. The invertase of corn radicle & its activity in successive stages of growth. Can. J. Botany 40: 113–26.
13. Putman, E. W. & W. Z. Hassid. 1954. Sugar transformation in leaves of Canna indica. I. Synthesis & inversion of sucrose. J. Biol. Chem. 207: 885–902.
14. Robinson, E. & R. Brown. 1952. The development of the enzyme compliment in growing root cells. J. Exptl. Botany 3: 356–74.
15. Sacher, J. A. & K. T. Glasziou. 1962. Regulation of invertase levels in sugar cane by an auxin-carbohydrate mediated control system. Biochem. & Biophys. Research Commun. 8: 280.
16. Solomon, A. K. 1953. In: Advances in Biological & Medical Physics, vol III, p 65. Academic Press, New York.
17. Wanner, H. & U. Leupold. 1947. Uber die longitudinale Verterlung der Saccarasktivitat in der Wurzelspitze. Ber. Schweiz. Botan. Ges. 57: 156–63.