# THE PHYSIOLOGY OF SUGAR-CANE

## V. KINETICS OF SUGAR ACCUMULATION

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*Summary*

Radioisotope techniques were used to study kinetics of sucrose, glucose, and fructose accumulation in slices of immature sugar-cane tissue. For all three sugars, accumulation rate was a hyperbolic function of sugar concentration, suggesting intermediate compound formation between the sugars and some receptor or "carrier" in the cell. Sucrose and glucose interacted competitively, implying that these two sugars (and probably also fructose, fructose 6-phosphate, and glucose 1-phosphate) shared the same carrier.

There appeared to be no interconversion of sucrose, glucose, and fructose before the rate-limiting step in accumulation. However, all sugars appeared in the tissue mainly as sucrose. This suggests that following the rate-limiting step in accumulation there may be a further step where the various sugars are converted to a common compound. A mechanism of sugar accumulation that could account for the above results is described.

## I. Introduction

In recent studies on sugar accumulation in sugar-cane (Bieleski 1960*a*, 1960*b*), the high concentration of endogenous sugar in the experimental material made it necessary to measure the rate of sugar accumulation in terms of the decrease in sugar concentration of the external solution. Because of the indirect nature of this method, it was also necessary to make estimates of the amounts of sugar held in the apparent free space (AFS), utilized in respiration, and converted into insoluble forms in order to establish that there was a true accumulation of sugar. The errors involved were such as to preclude any of the detailed quantitative work required in kinetic studies (Epstein and Hagen 1952). The requisite accuracy can only be obtained by using tissue with a very low sugar content, or by using radiotracer methods which allow the experimentally supplied sugar to be distinguished from that already present in the tissue.

Tracer techniques were therefore employed in an attempt to obtain more direct information on the factors involved in sugar accumulation by sugar-cane, and thereby to provide an indication of the type of mechanism involved in sugar accumulation.

## II. Materials and Methods

Immature cane tops were taken from a commercial crop, cv. Pindar, grown at Ingham, north Queensland, and were freighted by air to Sydney. The internodes

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selected were those in which elongation was not quite complete. The outer layer of sclerenchyma was split off, the parenchymatous core split in half, and cut transversely (by hand, with a razor-blade) into 1·4–1·6-mm thick slices. Disks 6·5 mm in diameter were cut from the slices with a sharp silver-steel cutter, washed for 16–20 hr in five or six changes of distilled water, blotted, and weighed into samples of 20 disks, 1·15–1·20 g. A surplus of disks was cut so that more uniform samples could be selected. Samples prepared in this way showed an average variation in accumulation rate of ±4% and a maximum of ±12%. The tracer compounds used were potassium [$^{36}$Cl]chloride and uniformly labelled [$^{14}$C]sucrose, [$^{14}$C]glucose, and [$^{14}$C]fructose supplied by the Radiochemical Centre, U.K.A.E.A., Amersham, England. The experimental solutions were made up as required with tracer, carrier sugar, and distilled water. Except in the experiments reported in Section II(*e*), the volumes of sugar solutions used were such that at the end of the experimental period, no individual sugar in the solution had altered in concentration by more than 10% of its original value. At the end of the absorption period, 24–36 hr, the solutions were drained from the disks, the disks washed for three 3-min periods in distilled water, and then aerated in three washes of distilled water, each at least 15 times the tissue volume, over the course of 1½ hr. In this way all traces of freely diffusible sugar held in the AFS were removed from the tissue. There can have been little or no reversible binding of sugar to sites in the AFS, since washing the tissue with carrier solutions removed no more $^{14}$C-labelled material than did washing with distilled water. Finally the tissue was blotted, placed in a sample tube, and 3 ml of 95% ethanol was added to give a final ethanol concentration of 70–75%. The tissue was crushed and from each sample three clear 0·50-ml aliquots were taken, plated on flat aluminium planchets, and dried under an infrared lamp. No spreading agent was needed, but the rim of the planchet was rubbed with silicone grease. Representative planchets were weighed before and after plating, and the plating thickness was found to be always less than 3·5 mg/sq. cm. Samples were counted to at least 1500 net counts, and the standard error was less than ±3·5%.

### (*a*) *Effect of Sugar Concentration on Accumulation Rate*

Solutions of sucrose, glucose, and fructose at various concentrations (20, 6·5, 2·0, 0·65, and 0·20 mg/ml; activity 0·01 μc/mg) were made up by serial dilution, and the tissue samples were allowed to accumulate sugar for a period of 24 hr. Samples were not duplicated.

### (*b*) *Interaction between Sugars during Accumulation from Mixtures*

A comparison was made of the rates at which tissue samples accumulated a $^{14}$C-labelled sugar in the absence and presence of another (unlabelled) sugar. The "accumulating" sugar ([$^{14}$C]sucrose, [$^{14}$C]glucose, or [$^{14}$C]fructose) was made into a solution of activity 0·1 μc/4 mg/ml, then aliquots were mixed either with an equal volume of distilled water, or with an equal volume of one of the "inhibiting" sugar solutions—glucose, fructose, and sucrose at 4 mg/ml and glucose 1-phosphate and fructose 6-phosphate at 0·022M (equivalent to 4 mg/ml free hexose). The rates at which tissue samples accumulated the $^{14}$C-labelled sugar from these mixtures were measured.

(*c*) *Competitive Inhibition of Sucrose Accumulation by Glucose*

[$^{14}$C]Sucrose solutions were made up at various concentrations (10·0, 2·0, 0·8, 0·4, and 0·25 mg/ml; activity 0·02 $\mu$c/mg) by serial dilution in distilled water or in 0·2, 0·4, or 0·8 mg/ml glucose solution. The length of the accumulation period was 33 hr, and the tissue samples were not duplicated.

(*d*) *Relationship between Sugar Accumulation and Salt Accumulation*

Salt uptake in mature and immature samples of sugar-cane storage tissue was studied by measuring the uptake of $^{36}$Cl from a 2·6 mM solution of potassium [$^{36}$Cl]chloride. Samples of 50 disks were taken, and chloride held in the AFS was not washed from the tissue. The tissue samples were blotted and then macerated by heating with dilute nitric acid at 100°C for 1 hr. The suspension volume was made to 20 ml, and a 10-ml aliquot of the suspension was counted in a liquid counter Geiger-Müller tube to at least 2000 counts. The effect of sucrose on chloride accumulation was determined by comparing the rates of $^{36}$Cl accumulation from a 3·0 mM potassium [$^{36}$Cl]chloride solution in the presence and absence of 30·0 mM sucrose. Samples of 50 disks were used as above.

In determining the effect of chloride on sucrose uptake, [$^{14}$C]sucrose solutions were made up at various concentrations (10·0, 2·0, 0·8, 0·5, and 0·25 mg/ml; activity 0·02 $\mu$c/mg) by serial dilution with distilled water or with 0·6 mM, 3·0 mM, or 15·0 mM KCl. The accumulation period was 33 hr, duplicate samples being used for all the sucrose treatments at concentrations 10·0 and 0·25 mg/ml, and single samples for the rest.

(*e*) *Chemical Identity of Accumulated Sugars*

Samples of tissue (10 disks, 0·55 g) were put into solutions of activity 3 $\mu$c/3 mg/3 ml of [$^{14}$C]sucrose, [$^{14}$C]glucose, [$^{14}$C]fructose, and "invert sugar" (equimolar [$^{14}$C]glucose and [$^{14}$C]fructose solutions), and allowed to accumulate sugar for 32 hr. Then the external solutions were taken, chilled, and made 5% with respect to trichloroacetic acid (TCA). The tissue samples were blotted, washed in three changes of distilled water (15 min in each), crushed in a chilled mortar with 2 ml ice-cold 5% TCA, and the residue extracted with a further 1 ml TCA. The extracts were centrifuged, the supernatants were made neutral to phenolphthalein with solid sodium carbonate, and stored at −20°C until required.

In preparation for chromatography, the excess salt was removed from samples of the extracts by shaking them with "Amberlite" IR-120 and IR-4B ion-exchange resins. The sugars were separated on Whatman No. 540 paper using ethyl acetate–propanol–water (55 : 32 : 13 v/v) as solvent. Rechromatography with carrier sugars was performed using Whatman No. 1 paper and the non-aqueous phase of n-butanol–acetic acid–water (50 : 10 : 40 v/v). Marker strips were run and the sugars were detected with alkaline silver nitrate. Untreated strips were scanned for radioactivity. A strip was cut into numbered segments, 8 mm wide, each of which was assayed for radioactivity with a thin end-window Geiger-Müller tube. Both sides of each segment were counted and the mean value taken. The relative radioactivity of each sugar was estimated by totalling the net counts for the appropriate segments.

To examine the extracts for sugar phosphates the precipitation procedure of LePage (cf. Umbreit, Burris, and Stauffer 1957) was employed, followed by descending chromatography on acid-washed Whatman No. 1 paper using n-propanol–ammonia–water (6 : 3 : 1 v/v) as solvent. Phosphates were detected with 0·1% ferric chloride–1% sulphosalicylic acid solution.

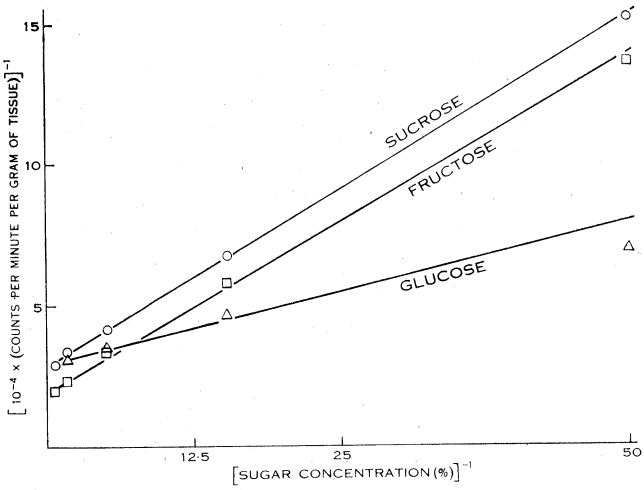



Fig. 1.—Double reciprocal plot of data showing variation of sugar-accumulation rate with sugar concentration. 1000 counts per minute per gram of tissue = sugar uptake of 1·5 mg/g.

*(f) Contribution of Vascular Bundles to Storage Tissue Sugar Accumulation*

Vascular bundles were present in the tissue samples used. To compare their accumulation rate with that of the surrounding parenchyma, 1 by 1 by 10 mm longitudinal strips of tissue were cut from an internode, one set being completely

TABLE 1

RATE CONSTANTS FOR SUCROSE, GLUCOSE, AND FRUCTOSE ACCUMULATION

| Sugar | $K_m$ | $V_{max.}$ |
|---|---|---|
| Sucrose | 0·85 mg/ml | 5·91 mg/g/day |
| Glucose | 0·31 mg/ml | 5·81 mg/g/day |
| Fructose | 1·23 mg/ml | 8·71 mg/g/day |

free of vascular tissue and the other set containing as much vascular tissue as possible. Samples of 1·50 g (approx. 130 pieces) were placed in solutions of [$^{14}$C]glucose of activity 0·1 $\mu$c/2 mg/ml, for 35 hr and the rates of sugar accumulation measured.

Radioautographs of tissue slices which had accumulated labelled sugar were made in the following manner. Transverse and longitudinal slices of tissue, 1 mm

thick, were allowed to accumulate sugar for 36 hr from a solution of [$^{14}$C]glucose of activity 0·5 $\mu$c/0·5 mg/ml; they were washed in four changes of distilled water over 2 hr. The slices were blotted dry, fixed to "no-screen" X-ray film (Ilford Industrial G) with adhesive tape, and immediately covered with powered dry ice. The material was kept in dry ice, at −78°C, for 2 days, then the tissue was removed and kept frozen while the film was developed. The short range of $^{14}$C $\beta$-particles (maximum 0·3 mm in plant tissue) permitted sufficiently high resolution, even though 1 mm slices of tissue were used. It was not possible to use very thin tissue

TABLE 2

SUPPRESSION OF ACCUMULATION OF ONE SUGAR BY ANOTHER

| Accumulating Sugar | Replicates | Time (hr) | Relative Accumulation Rate | Interacting Sugar |
|---|---|---|---|---|
| [$^{14}$C]Sucrose, 5·8 mM | 3 | 25 | 1·00 | None |
| | 3 | 25 | 0·34 | Glucose, 11 mM |
| | 3 | 25 | 0·85 | Fructose, 11 mM |
| [$^{14}$C]Fructose, 11 mM | 3 | 25 | 1·00 | None |
| | 3 | 25 | 0·45 | Glucose, 11 mM |
| | 3 | 25 | 0·85 | Sucrose, 5·8 mM |
| [$^{14}$C]Glucose, 11 mM | 3 | 25 | 1·00 | None |
| | 3 | 25 | 0·90 | Sucrose, 5·8 mM |
| | 3 | 25 | 0·92 | Fructose, 11 mM |
| | 2 | 29 | 0·77 | Glucose 1-phosphate, 11 mM |
| | 2 | 29 | 0·69 | Fructose 6-phosphate, 11 mM |

sections, as all soluble contents of the cell would have been lost. Chemical fogging of the film was not serious, as the same results were obtained when the tissue was separated from the film by a thin sheet of mica.

## III. RESULTS

### (*a*) *Effect of Sugar Concentration on Accumulation Rate*

For each of the three sugars, sucrose, glucose, and fructose, the plot of accumulation rate against sugar concentration gave a hyperbola of the form

$$y = cx/a(x+a).$$

Thus a double reciprocal plot of the data (Lineweaver and Burk 1934) gave a straight line cutting the $y$-axis for each sugar (Fig. 1). From the $y$-axis intercept and slope of each line, the characteristic $V$ (maximum velocity of accumulation at infinite concentration) and $K_m$ (concentration where accumulation rate equals $\frac{1}{2}V$) can be calculated for each sugar (Table 1).

*(b) Interaction between Sugars during Accumulation from Mixtures*

It was found that there was an interaction between the various sugars during their accumulation (Table 2). Glucose, showing the lowest $K_m$, was least affected of the accumulated sugars, and most effective of the inhibiting sugars.

*(c) Competitive Inhibition of Sucrose Accumulation by Glucose*

The kinetics of the interaction between sucrose and glucose were studied by measuring the uptake rate of [$^{14}$C]sucrose at various concentrations in the presence of glucose at various concentrations. When the data were expressed on a double reciprocal plot, they gave a family of straight lines which had the same intercept on the $y$-axis (same $V$) but different slopes (Fig. 2), indicating that the inhibition was a competitive one.

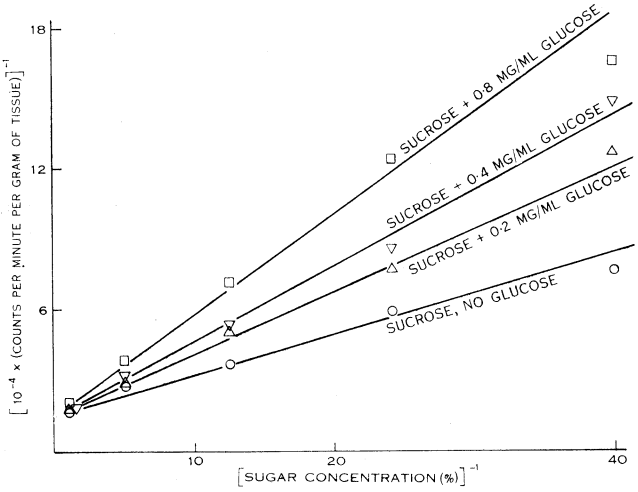



Fig. 2.—Double reciprocal plot of data showing competitive inhibition of sucrose uptake by glucose. 1000 counts per minute per gram of tissue = sucrose uptake of 0·73 mg/g.

*(d) Relationship between Sugar Accumulation and Salt Accumulation*

The uptake of chloride by immature sugar-cane storage tissues appeared to conform to the pattern of chloride accumulation by other parenchymatous tissues. There was an initial rapid uptake into the AFS, followed by a slower uptake continuing to a tissue concentration at least eight times that in the external solution (Fig. 3). When the tissues were washed in 0·01M potassium chloride carrier, [$^{36}$Cl]chloride equivalent only to the first stage of uptake could be recovered in solution. Mature sugar-cane storage tissues accumulated chloride much more slowly. The rates of salt accumulation in tissues of two different ages were comparable to the rates of sugar accumulation (Table 3).

The effects of sugar on salt accumulation and of salt on sugar accumulation were determined. Sucrose at 10 times the chloride concentration—30 mM as compared with 3·0 mM—did not affect the rate of chloride uptake. However, potassium chloride

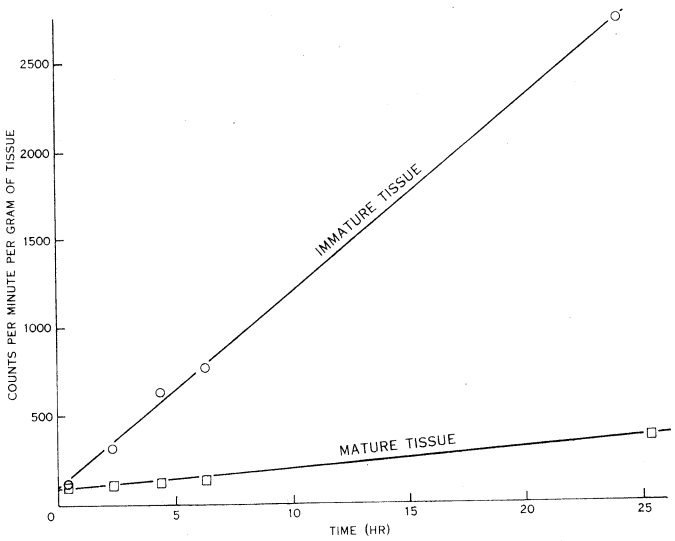



Fig. 3.—Chloride uptake by mature and immature tissue in 2·6 mM potassium [$^{36}$Cl]chloride. 1000 counts per minute per gram of tissue = chloride uptake of 6·5 μmoles/g.

at various concentrations inhibited sucrose uptake slightly. When the data were expressed on a double reciprocal plot as before, the family of straight lines obtained

TABLE 3

RELATION OF SALT ACCUMULATION TO SUGAR ACCUMULATION

| Tissue | External Solution* | Accumulation Rate |
|---|---|---|
| Immature | K$^{36}$Cl, 2·6 mM | 17·0 μmoles/g/day |
| | Sucrose, 14 mM | 13·5 μmoles/g/day |
| | | (4·6 mg/g/day) |
| Mature | K$^{36}$Cl, 2·6 mM | 1·8 μmoles/g/day |
| | Sucrose, 14 mM | 5·9 μmoles/g/day |
| | | (2·0 mg/g/day) |

* Concentrations were chosen to give accumulation rates in immature tissue within three-quarters of their saturation values.

had slightly different slopes but slightly different intercepts on the $y$-axis (Fig. 4), indicating that the slight inhibition was probably non-competitive.

### (e) *Chemical Identity of Accumulated Sugars*

When sugars which had been accumulated by sugar-cane tissue slices were extracted, chromatographed, and identified, it was found that regardless of the identity of the radioactive sugar supplied, the major radioactive product in the tissue was sucrose (Table 4). In the first experiment radioactivities were low, and it was not possible to separate accurately the activity due to glucose from that due to fructose. In addition, small amounts of sodium trichloroacetate in the extract caused some material to be left at the chromatogram origin. In the second experiment, better separation was obtained by chromatographing the extracts, cutting out and

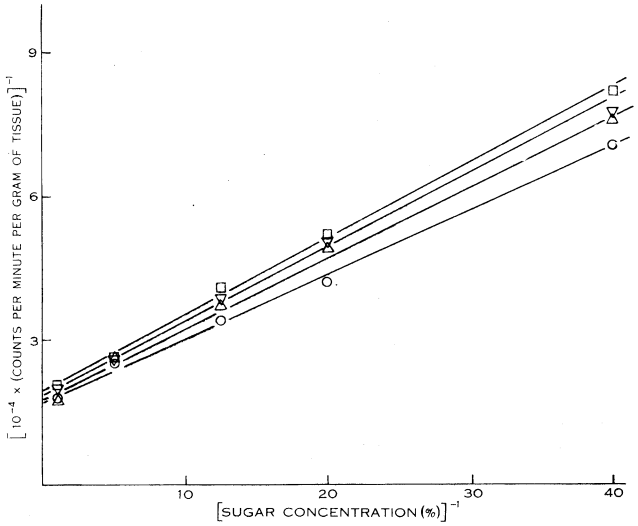



Fig. 4.—Double reciprocal plot of data showing inhibition of sucrose uptake by chloride. ○ Sucrose, no chloride. △ Sucrose + 0·6 mM KCl. ▽ Sucrose + 3·0 mM KCl. □ Sucrose + 15·0 mM KCl. 1000 counts per minute per gram of tissue = sucrose uptake of 0·72 mg/g.

discarding the TCA area, and rerunning the remaining spots with carrier sugar in a second solvent system. As before, the main $^{14}C$ activity was due to sucrose; glucose and fructose were present in smaller and approximately equal amounts (Table 4). Material X was an unknown compound with an $R_F$ similar to that of xylose, and material A, which reacted with ninhydrin, was probably an amide. Any sugar phosphates present were below the limits of detection.

Sugars present in the external solution at the end of the experiment were separated and identified (Table 5). It was found that when sucrose was supplied to the tissue, small amounts of glucose and fructose appeared in the external solution, indicating that some hydrolysis might have occurred. However, when glucose or fructose were supplied, small amounts of radioactive sucrose and of fructose or glucose also appeared in the external solution. Thus there must have been some outward movement of sugars from the tissue, even though there was a net accumulation taking place: a similar phenomenon has been observed for other active transport

processes (Wilbrandt, Frei, and Rosenberg 1956; Briggs 1957). When invert sugar was supplied, it was found that at the end of the experiment there was much less glucose than fructose present in the external solution, indicating that glucose had

TABLE 4

CHROMATOGRAPHIC SEPARATION OF ACCUMULATED SUGARS

| Expt. No. | Sugar Supplied at 1 mg/ml | Total Sugar Accumulated* (mg/g) | Percentage of Total $^{14}C$ Activity of Extract in: | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Origin | Raffinose | Sucrose | Glucose | Fructose | A | X |
| 1 | [$^{14}C$]Sucrose | — | 6 | — | 61 | 33 | | — | — |
| | [$^{14}C$]Glucose | — | 4 | — | 60 | 36 | | — | — |
| | [$^{14}C$]Fructose | — | 7 | — | 61 | 32 | | — | — |
| | [$^{14}C$]Invert sugar† | — | 5 | — | 58 | 37 | | — | — |
| 2 | [$^{14}C$]Sucrose | 2·2 | Trace | Trace | 63 | 17 | 16 | 2 | 1 |
| | [$^{14}C$]Glucose | 3·3 | Trace | Trace | 57 | 21 | 18·5 | 1·5 | 1 |
| | [$^{14}C$]Fructose | 1·9 | Trace | Trace | 59 | 17·5 | 16·5 | 3 | 2 |
| | [$^{14}C$]Invert sugar | 3·1 | Tface | Trace | 57 | 19 | 19 | 2 | 2 |

* Estimated from $^{14}C$ activity of alcohol extract, corrected for self-absorption: values ±8%.

† Equimolar glucose plus fructose, each 0·5 mg/ml.

been preferentially absorbed from the mixture (Table 5). This is what would be expected from the results reported in the previous sections.

TABLE 5

EFFECT OF ACCUMULATING TISSUE ON COMPOSITION OF EXTERNAL SOLUTION

| Sugar Supplied at 1 mg/ml | Amount Absorbed by Tissue (%) | Amount (mg/ml) in Final Solution of: | | |
|---|---|---|---|---|
| | | Sucrose | Glucose | Fructose |
| [$^{14}C$]Sucrose | 42 | 0·418 | 0·069 | 0·093 |
| [$^{14}C$]Glucose | 65 | 0·039 | 0·292 | 0·018 |
| [$^{14}C$]Fructose | 37 | 0·059 | 0·096 | 0·475 |
| [$^{14}C$]Invert sugar | 61 | 0·051 | 0·076 | 0·263 |

### (*f*) *Contribution of Vascular Bundles to Storage Tissue Sugar Accumulation*

The storage tissue slices used in the previous experiments contained a constant ratio of 92% storage parenchyma, 7·5% xylem (vessels, parenchyma, and fibres, including phloem fibres), and 0·5% phloem sieve tubes, as measured from microscope sections. The sample which was cut to include as much vascular tissue as possible

contained 20% vascular tissue and 80% storage parenchyma, and accumulated glucose at almost twice the rate of the sample of pure storage parenchyma (Table 6). If the parenchyma tissue present in the vascular bundle sample accumulated glucose at the same rate as the parenchyma in the parenchyma sample, the vascular bundles alone must have accumulated glucose at five times that rate.

The radioautographs of slices of storage tissue verified that sugar accumulation was considerably more rapid in the vascular bundles. Microscopic comparison of each section with its radioautograph revealed that the xylem parenchyma accumulated sugar at approximately the same rate as the storage parenchyma, and that the phloem, excluding the fibres, was responsible for much of the increased rate of sugar accumulation in the vascular bundles. Estimates of film density and silver grain counts indicated that sugar accumulation in the phloem occurred at a rate at least 10 times and possibly 40 times that in the storage parenchyma.

TABLE 6

RATES OF SUGAR ACCUMULATION IN PARENCHYMA AND STORAGE TISSUE

| Tissue Sample | Parenchyma (%) | Vascular Tissue (%) | Sugar Accumulation Rate (mg/g/day) |
|---|---|---|---|
| Parenchyma | 100 | 0 | 2·44 |
| Parenchyma | 100 | 0 | 2·63 |
| Vascular | 80 | 20 | 4·32 |
| Vascular | 80 | 20 | 4·70 |

## IV. DISCUSSION

It has already been shown (Bieleski 1960*a*) that about 90% of the sugar taken up by sugar-cane tissue is accumulated as sugar, and only about 10% is utilized in other processes, mainly respiration. The data in Section III(*e*), which also show that most of the sugar taken up is accumulated, provide the best evidence to date that a true active sugar transport process is operating in immature sugar-cane storage tissues. They show that [$^{14}$C]sucrose, supplied at an initial concentration of 1·0 mg/ml and a final concentration of 0·42 mg/ml (Table 5) could be recovered as sucrose from the non-free space of the tissue at an overall concentration of 1·40 $\pm$0·15 mg/g (from Table 4: 63% of 2·2 mg/g) when the endogenous sucrose concentration was 41 mg/g. A moderate contribution to the total sugar uptake measured was made by the small amounts of phloem tissue present in the samples. Phloem, which accumulated sugar at about 20 times the rate of the storage parenchyma (3–6 mg/g/day), must therefore have accumulated the considerable amount of 80 mg sugar/g phloem tissue over a 24-hr period. The ratio of the accumulation rates is of the same order as the ratio of the respiration rates of the two tissues, 400–1000 $\mu$l $O_2$/g/hr for phloem

tissues (Duloy and Mercer 1961; Bieleski, unpublished data) and 30–40 $\mu$l $O_2$/g/hr for immature sugar-cane storage parenchyma. It is therefore felt that the same process, which operates more rapidly in the more actively metabolizing tissue, is responsible for sugar accumulation in these two different tissues. The activity of this sugar-accumulation process in phloem tissues suggests that it may be involved in or related to the actual phloem transport process (Arisz 1952).

The kinetics of sugar accumulation in sugar-cane can be examined. If the rate of accumulation were limited by diffusional movement of sugar into the tissue, the uptake rate would be directly proportional to the external sugar concentration. The hyperbolic relationship actually observed can best be explained on the basis of some type of reversible complex formation (cf. Cohen and Monod 1957):

$$\text{sugar} + \text{receptor} \rightleftharpoons \text{sugar–receptor complex.}$$

Such a generalized equation would, for example, describe the adsorption of sugar to some surface site (Langmuir adsorption kinetics), or the reversible combination of sugar with some permanent receptor site in the cell. However, these phenomena are limited in time, and do not explain how a continuous accumulation process can operate. Alternatively, the initial sugar–receptor complex can be a transitory intermediate (see Epstein and Hagen 1952; also Epstein and Leggett 1954):

$$\text{sugar} + \text{receptor} \rightleftharpoons \text{sugar–receptor} \rightarrow \text{sugar product} + \text{receptor.}$$

On this view, the receptor has a catalytic function. The reaction is thus closely analogous to that between an enzyme and its substrate:

$$\text{substrate} + \text{enzyme} \rightleftharpoons \text{substrate–enzyme complex} \rightarrow \text{product} + \text{enzyme,}$$

in which the rate of reaction can be expressed as the straight-line relationship

$$\frac{1}{v} = \frac{K_m}{V} \cdot \frac{1}{s} + \frac{1}{V},$$

where

$v$ = rate of reaction,
$V$ = maximum rate of reaction at infinite concentration,
$s$ = concentration of substrate, and
$K_m$ = dissociation constant of the enzyme–substrate complex.

This type of straight-line relationship was found to hold for sugar accumulation in sugar-cane, where $v$ = rate of accumulation, $s$ = sugar concentration, and $V$ = rate of sugar accumulation at infinite sugar concentration.

The finding that tissues which accumulated sugars rapidly were also capable of rapid salt accumulation suggested that there might be some interaction between the two processes. Sugars are known to affect salt accumulation in some plant tissues (Arisz and Sol 1956) but there was no such effect in sugar-cane. The presence of potassium chloride gave slight inhibition of sucrose uptake, the data conforming to the relationship characteristic of non-competitive inhibition (Epstein and Hagen 1952),

$$\frac{1}{v} = \left(1 + \frac{I}{K_i}\right)\left(\frac{1}{V} + \frac{K_m}{V} \cdot \frac{1}{s}\right),$$

where $K_i$ is the dissociation constant of the inhibitor–receptor complex, $I$ is the concentration of the inhibitor, and other symbols as before. Because of the small differences involved, the data do not firmly establish this relationship. However, since the effects were small, despite the high concentrations of potassium chloride used, it can be concluded that chloride accumulation and sugar accumulation in sugar-cane are essentially independent processes.

There was an interaction between the various sugars during their accumulation (cf. Harley and Smith 1956; Riklis, Haber, and Quastel 1958). Glucose 1-phosphate was considerably more effective than either sucrose or fructose in inhibiting glucose accumulation, yet it has previously been shown that glucose 1-phosphate is not accumulated by sugar-cane (Bieleski 1960*a*). A similar phenomenon has been observed by Thimann and Marre (1954), who found that sugar phosphates were not taken up by *Avena* coleoptiles, yet inhibited sucrose and glucose utilization. This type of inhibition and structural specificity is another point of similarity between the sugar-accumulation process and enzyme processes. Glucose, with a high affinity for the receptor ($K_m = 0{\cdot}31$ mg/ml) strongly inhibited accumulation of sucrose and fructose, which had lower affinities ($K_m = 0{\cdot}85$ mg/ml and $1{\cdot}23$ mg/ml respectively). Glucose accumulation was not inhibited by sucrose or fructose to the same extent. This contradicts a previous result (Bieleski 1960*a*) which indicated that sucrose almost completely inhibited glucose accumulation. The earlier experiment was carried out using an indirect method of measuring sugar uptake, but it is unlikely that the inherent errors of the method can account for the complete difference of behaviour observed. In other tissues pretreatment conditions (Loughman 1960) and season of growth (Hanson and Kahn 1957) are known to affect accumulation behaviour.

When the inhibition of sucrose accumulation by glucose was examined in more detail, it was found that the inhibition conformed to the relationship

$$\frac{1}{v} = \frac{1}{V}\left(K_m + \frac{K_m \cdot I}{K_i}\right)\frac{1}{s} + \frac{1}{V},$$

where the symbols have the same meaning as before. This relationship is characteristic of a competitive inhibition for a common receptor (cf. Epstein and Hagen 1952). The values of $K_i$ give a measure of the affinity of the inhibitor for the receptor. The values of $K_i$ for each of the three concentrations of glucose employed were found to be very similar to the $K_m$ values for glucose alone (Table 7). Again, this is what would be expected if the two sugars were competing for the same receptor.

The evidence suggests that sucrose is absorbed as such. If sucrose uptake were dependent on its hydrolysis, then either the sucrose hydrolysis would be rate-limiting, and the uptake rate would not be an hyperbolic function of concentration; or else the sucrose would have been accumulated as if it were a mixture of glucose and fructose. Neither of these two conditions was observed (Fig. 1; Table 5). Instead, each sugar showed a characteristic dissociation constant, $K_m$, and a characteristic rate constant $V$. Also, glucose inhibited sucrose uptake in a manner completely

consistent with these data. This indicates that up until the rate-limiting step in accumulation the three sugars are accumulated without significant interconversion. These results support the opinion of Street and Lowe (1950) that plant tissues are capable of utilizing sucrose directly, and that where sucrose appears to be absorbed only as its hydrolysis products, an independent inversion process may allow subsequent glucose absorption to inhibit or mask a true sucrose absorption process (see also Harley and Smith 1956).

On the other hand, regardless of the sugar supplied to the tissue, the major accumulation product was [$^{14}$C]sucrose. [$^{14}$C]Glucose and [$^{14}$C]fructose were present in smaller and approximately equal amounts, suggesting that they may have originated as secondary hydrolysis products of sucrose. These results demonstrate that after the rate-limiting step there is an interconversion of the various sugars.

TABLE 7

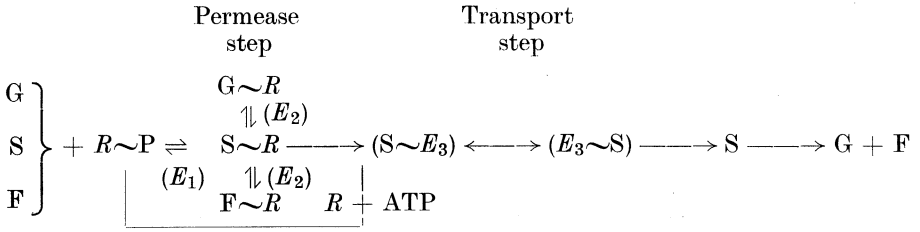
"DISSOCIATION CONSTANT" OF GLUCOSE AS AN INHIBITOR OF SUCROSE ACCUMULATION

| Accumulating Sugar | Inhibiting Sugar | $K_m$ or $K_i$ Value |
|---|---|---|
| Glucose | — | $K_m$ (glucose) 0·34 mg/ml |
| Sucrose | — | $K_m$ (sucrose) 1·02 mg/ml |
| Sucrose | Glucose, 0·2 mg/ml | $K_i$ (glucose) 0·35 mg/ml |
| Sucrose | Glucose, 0·4 mg/ml | $K_i$ (glucose) 0·40 mg/ml |
| Sucrose | Glucose, 0·8 mg/ml | $K_i$ (glucose) 0·44 mg/ml |

The kinetic pattern of the sugar-accumulation process in sugar-cane closely resembles that of other accumulation processes. In particular it resembles the sugar-accumulation process in yeast (Rothstein 1954) and bacteria (Monod 1956; Rickenberg *et al.* 1956), even showing comparable $K_m$ values. These authors have provided a considerable body of evidence supporting their claim that the "receptor" involved is a specific inducible enzyme, which they term a "permease". They show that the *Escherichia coli* cell contains several permeases, each of which is responsible for the accumulation of a particular class of compounds: galactosides, glucosides, glucuronides, and amino acids (Cohen and Monod 1957). The characteristics of sugar accumulation in sugar-cane are consistent with the operation of one such permease.

Possible mechanisms operating in the sugar-accumulation process can be considered. The most significant points are as follows: (1) The rate-limiting reaction in the process has the characteristics of an enzyme reaction. (2) Up to and including the rate-limiting step, the various sugars (sucrose, glucose, and fructose) are distinct, each having its own $K_m$ value and interacting competitively with the other sugars. Glucose is the most efficiently accumulated sugar. (3) A subsequent step in accumulation is such that sucrose is the major or only accumulation product, regardless of the sugar supplied (see also Glasziou 1960). One possibility is that all sugars are

converted to a common compound during their accumulation. This final step need not be a part of the actual accumulation process. However, in view of the likelihood that sugar-accumulation, nectary-secretion, and phloem transport processes are closely related, and in view of the universal occurrence of sucrose as the major sugar in all these processes, the view adopted here is that the sucrose-formation step is an integral part of the accumulation mechanism. The question then arises as to why glucose is preferentially taken up, and not sucrose. However, if a labile sucrosyl complex were the form in which the carbohydrate moves across the permeability barrier(s), and the sucrose appearing in the tissue was a breakdown product of the complex, glucose could be preferentially used in the synthesis of the complex. Accumulation could therefore occur in the following way:




The first step is an enzyme ($E_1$)-catalysed linking of the various sugars to one activated receptor ($R\sim$P). The step is rate-limiting, and has the characteristics of a permease. In the second enzyme ($E_2$)-catalysed step, the "activated" sugars are interconverted. In the third step, activated sucrose is passed to the surface of a membrane-located enzyme ($E_3$) of the type proposed by Mitchell (1957) and Mitchell and Moyle (1958). There is a subsequent transfer of the sucrosyl group to a different position on the enzyme surface, on the inner side of the membrane. In this way group transfer would give the actual transport step. Finally sucrose is released to the vacuole where it may form glucose and fructose by hydrolysis. Cohen and Monod (1957) equated the permease-catalysed reaction to the accumulation step, but noted that (p. 176) "the system may comprise a sequence of two (or more) . . . enzyme-catalysed . . . successive steps in the entry reaction"; this is the view taken here. This scheme is similar to one, based on a different experimental approach, described by Porter and May (1955) to account for various aspects of the metabolism of sugars by tobacco leaf disks. Further evidence bearing on a mechanism of this type, also for sugar-cane, has been discussed by Glasziou (1960). The differences between his scheme and the one described here would be less fundamental if the fructose in the inner space of the sugar-cane cell were not in a single pool, as assumed by Glasziou, but in at least two independent pools. Independent inner space pools were suggested to occur for sucrose (Glasziou 1960) and have been demonstrated for glucose in potato (Laties 1962).

The postulated behaviour of the $R\sim$P compound is similar to the known behaviour of uridine triphosphate. Uridine compounds are known to be involved in carbohydrate metabolism in sugar-cane. More definitive experiments are needed to establish whether they also play a central role in the sugar-accumulation process.

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