IV. EFFECTS OF INHIBITORS ON SUGAR ACCUMULATION IN STORAGE TISSUE SLICES

By R. L. Bieleski*

[Manuscript received February 8, 1960]

Summary

Various metabolic inhibitors, at pH 5.5, affected sugar accumulation in immature sugar-cane storage tissues. The rate of accumulation was reduced by 10^{-4} M mercuric ion, 10^{-4} M p-chloromercuribenzoate, cyanide, and cupric ion, and 2 \times 10^{-3} M phloridzin. Accumulation was completely inhibited and sugar leakage induced by 10^{-4} M dinitrophenol, 10^{-4} M mercuric ion, and 10^{-2} M p-chloromercuribenzoate, cyanide, cupric ion, azide, arsenate, and iodoacetate. The effects of 10^{-4} M dinitrophenol and 10^{-4} M cyanide were reversible, but that of 10^{-3} M cyanide was irreversible. Only slight effects were produced by borate, phosphate, and magnesium ion.

The behaviour of sugar-cane sugar accumulation towards inhibitors was similar to that of other transport mechanisms, being somewhat more sensitive than most plant processes and less than animal ones. It differed from sugar accumulation in animal tissues in showing no specific sensitivity to phloridzin. The results suggest that sugars are contained within the immature storage cell by the continuous operation of a metabolic storage mechanism rather than by an impenetrable barrier to sugar diffusion.

I. INTRODUCTION

It has been shown that sugars can be taken up against a concentration gradient into sugar-cane storage tissues through the operation of an active accumulation mechanism (Bieleski 1960). This was found to have features in common with other accumulation mechanisms (Brown and Danielli 1954; Robertson 1956) and appeared closely allied to various active phloem translocation processes (Esau, Currier, and Cheadle 1957). Several metabolic inhibitors have been shown to inhibit these processes. The most widely used inhibitor has been 2,4-dinitrophenol (DNP), 10^{-5}-10^{-3} M, which has been found by various authors to inhibit phosphate and sugar transport in the phloem; sugar uptake in yeast, in bacteria, and in animal cells; salt accumulation in storage tissues, in algal tissues, and in yeast cells; amino acid accumulation; auxin uptake and translocation; and streptomycin uptake by *Nitella* cells. Partially or completely anaerobic conditions have also been shown to affect a wide variety of such processes. The other inhibitors used fall into two main groups: respiration inhibitors, such as cyanide and carbon monoxide; and sulphydryl (-SH) group inhibitors, such as cupric ion, mercuric ion, p-chloromercuribenzoate (PCMB), and iodoacetate (James 1953a; 1953b). A considerable body of evidence indicates that the glycoside phloridzin (phlorrhizin, phlorizin, phlorrhizin), 10^{-6} -10^{-5} M, can act as a specific inhibitor of sugar uptake in animal

* Botany Department, University of Sydney: present address, Fruit Research Division, D.S.I.R., Auckland, N.Z.
cells (LeFevre 1948; Wilbrandt 1954; Crane, Field, and Cori 1957; Riklis and Quastel 1958b); though in higher concentrations it also appears to act as an inhibitor of phosphate transfer (James 1953a).

Despite the wide variety of accumulation and translocation systems which have been studied, a consistent pattern of behaviour to the various inhibitors has been found. Transport was largely or completely inhibited by $10^{-4}$M DNP, and in some cases there was an outward "leakage" of solutes. Anaerobic conditions, $10^{-3}$M potassium cyanide, and (in plant tissues) 95 per cent. carbon monoxide in the dark all caused a partial or complete inhibition of active transport. Most of the processes were found to be extremely sensitive to $-SH$ inhibitors. Phloridzin in concentrations as low as $10^{-6}$M was an effective inhibitor of sugar accumulation in animal tissues of various kinds. There is little information on the effect of phloridzin on plant tissues.

Only a few attempts have been made to increase accumulation and translocation rates by adding various substances to the medium. Borate has been claimed to increase the rate of translocation of sugars in soybean (see Dugger, Humphreys, and Calhoun 1957), potassium ion to stimulate glucose uptake in animal intestines (Riklis and Quastel 1958a, 1958b), and calcium, magnesium, and manganese ions to stimulate sugar uptake in yeast cells (Rothstein 1954).

This paper describes the effects of a range of inhibitors on the process of sugar accumulation in the storage tissues of sugar-cane. It conformed to the general pattern of behaviour of active transport systems towards inhibitors. Various compounds were tested for their ability to increase the rate of sugar uptake.

![Graph showing inhibition of sugar accumulation in immature tissues by DNP](image-url)

Fig. 1.—Inhibition of sugar accumulation in immature tissues by DNP, and its reversal by washing: ⌋ 2 per cent. sucrose; △ 2 per cent. sucrose, made to $10^{-5}$M DNP by addition of DNP at hour 37. Both samples placed in distilled water at hour 78. Tissue sample weight 40 g; solution volume 120 ml; water volume 120 ml. Treatments in duplicate; mean values plotted, individual values agreeing to within $±0.2$ mg/g fresh weight.
II. Materials and Methods

The sugar-cane used, cv. Pindar, was cut from a commercial crop grown at Ingham, N. Qld., and sent by air-freight to Sydney. Mature and immature internodes were selected as previously described (Bieleski 1960) and slices of tissue 1·2-1·7 mm thick cut from the storage parenchyma as before. The slices were washed in four to six changes of distilled water over 15-20 hr, then blotted dry. Weighed samples, generally 60 g (240-300 slices), were placed in aerated sugar solutions, usually 0·25 per cent. glucose, and the rate of sugar accumulation or leakage was measured in terms of sugar concentration of the external solution.

![Graph showing sugar uptake in mature and immature tissues](image)

Fig. 2.—Inhibition of sugar accumulation in mature tissues by DNP, and its reversal by washing: △ mature tissue in 0·5 per cent. sucrose; ▲ mature tissue in 0·5 per cent. sucrose +10⁻⁴M DNP; ○ immature tissue in 0·5 per cent. sucrose; ● immature tissue in 0·5 per cent. sucrose +10⁻⁴M DNP. At hour 13 mature tissue samples removed from sugar solutions, washed in four changes of distilled water over 10 hr, then replaced in fresh 0·5 per cent. sucrose solution without DNP. Immature samples discarded at hour 23 since they showed signs of damage or infection. Tissue sample weight 70 g; solution volume 140 ml, water volume 200 ml. Duplicate samples used in all treatments; mean values plotted, individual values agreeing to within ±0·2 mg/g fresh weight.

Aliquots of the external solution were taken at various times, and sugars estimated by the method of Somogyi (1945). The effect of a compound was determined either by comparing the rates of sugar accumulation in a single sample before and after adding the inhibitor (e.g. Fig. 1) or by comparing the accumulation rates in a control sample and a sample to which the compound to be tested had been added (e.g. Fig. 2). Buffers were unnecessary and undesirable (Bieleski 1960) as the tissue kept the solution at pH 5·2-5·7 throughout all experiments. Inhibitor solutions were adjusted to pH 5·5 before use. Where particularly volatile inhibitors such as cyanide were used, closed-circuit aeration was employed to avoid loss of the inhibitor. The volume of air included in the circuit was such that the tissue respiration during the experiment did not lower the oxygen concentration of the air.
below 0.190 atm (oxygen concentration of air 0.205 atm at start of experiment), while at the same time the inhibitor concentration in solution was substantially unaltered.

III. RESULTS

(a) Inhibition by Dinitrophenol

Sugar accumulation in mature and immature tissues was completely inhibited by $10^{-5}$ M DNP; and endogenous sugars were slowly lost from the tissue to the external solution (Figs. 1 and 2). When the inhibited tissue was placed in distilled water, the sugar leakage continued for about 30 hr, but eventually the tissue recovered from the inhibition and commenced to re-accumulate the sugar which had been lost to solution (Fig. 1). When the inhibited tissue was washed in several changes of distilled water and then replaced in fresh dilute sugar solution, the recovery was more rapid and complete (Fig. 2).

It appeared possible that the loss of sugar from the tissue during DNP inhibition might occur because a normal slow diffusion of sugar through the tonoplast (under the influence of the high concentration gradient) was no longer being opposed by the energy-requiring accumulation mechanism. If this were so, the rate of sugar leakage from mature and immature tissues under various conditions might yield some information on the relative permeability of their cell tonoplasts. Although in any one experiment the replicates agreed well on the relative effects of DNP on mature and immature tissues, results varied from one experiment to another. The following example illustrates some of the factors involved.
The effect of a range of DNP concentrations on the rate of sugar leakage from mature and immature tissue slices was studied (Fig. 3). Over the first 5 hr the two types of tissue were affected in the same way by the DNP. The rate of leakage was several times greater from the mature tissue, and in both tissues it was about 10 times greater in \(2 \times 10^{-4}\)M DNP than in distilled water. Compared with the first 5 hr, the rates of leakage during the next 7 hr and during the following 11 hr were lower for the lower DNP concentrations, and higher for the higher concentrations. Sugar leakage became very rapid from mature slices in solutions above \(1 \times 10^{-3}\)M DNP, and from immature slices in solutions above \(5 \times 10^{-5}\)M DNP. The most dilute DNP solutions, particularly when bathing the mature tissue, showed a definite tendency to lose their characteristic yellow colour, indicating that the tissue may have been inactivating or absorbing the DNP.

**(b) Inhibition by Cyanide, Azide, and Arsenate**

Potassium cyanide, \(10^{-3}\)M, completely inhibited sugar accumulation and induced a rapid leakage of sugar from the tissue (Fig. 4). Though glucose was the only sugar supplied in the solution originally, 74 per cent. of the sugar leaking out from the tissue was non-reducing sugar (presumably sucrose) and only 26 per cent. was reducing sugar. Thus the leaking sugar may have come mainly from the endogenous sugar in the cell vacuole, the accumulated sugar may have been converted to sucrose during its accumulation, or more probably both factors may have been involved. In the control sample, a small amount of non-reducing sugar also appeared in the external solution over the course of the experiment, indicating that there
was a small outward movement of sugar from the tissue even while there was a net accumulation of total sugar. When the tissue was washed in several changes of distilled water then returned to fresh dilute sugar solution, there was no accumulation, and leakage continued at a reduced rate. Hence, unlike anaerobic inhibition (Bieleski 1960) and $10^{-3}$M DNP inhibition, the $10^{-3}$M cyanide inhibition was not readily reversible. In a $10^{-4}$M solution, cyanide also completely inhibited sugar accumulation, and induced a slight sugar leakage which ceased after 3 hr. When the tissue was washed in several changes of distilled water then returned to fresh sugar solution, the accumulation recommenced at approximately the original uninhibited rate (Fig. 5).

Sodium azide and sodium arsenate, each at a final concentration of $10^{-3}$M, both completely inhibited sugar accumulation and induced rapid sugar leakage from the tissue (Table 1).

(c) Inhibition by Sulphydryl Group Inhibitors

Sodium iodoacetate, $10^{-3}$M, completely inhibited sugar accumulation and caused rapid sugar leakage (Table 1). Mercuric ion (mercuric chloride) caused no inhibition of sugar accumulation in sugar-cane tissues when at $10^{-6}$M, a concentration which is known to affect sugar accumulation in animal tissues. At $10^{-5}$M, mercuric ion caused a slight (5-10 per cent.) reduction in the rate of sugar accumulation; while at $10^{-4}$M, inhibition was complete, and a rapid and irreversible sugar leakage resulted (Table 1). PCMB is a more specific inhibitor of -SH groups than mercuric ion. At $10^{-4}$M, PCMB caused a 0-10 per cent. reduction in the rate of sugar accumulation, and at $10^{-3}$M (the concentration usually used for inhibition of -SH groups) PCMB completely inhibited sugar uptake and caused sugar leakage
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(Table 1). Cupric ion (cupric sulphate), which often appears to act in a similar fashion to PCMB, at $10^{-4}$M reduced the rate of sugar accumulation up to 20 per cent., and at $10^{-5}$M caused complete inhibition and induced sugar leakage (Table 1).

(d) Inhibition by Phloridzin

Phloridzin, at concentrations completely effective in inhibiting sugar accumulation in animal tissues ($10^{-6}$-$10^{-5}$M), had no effect on sugar accumulation in sugar-cane. At $2 \times 10^{-3}$M, phloridzin caused a 10–80 per cent. reduction in the rate of sugar accumulation (Fig. 6).

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Molarity</th>
<th>Inhibition (%)</th>
<th>Leakage ( % uptake)</th>
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<tbody>
<tr>
<td>Azide</td>
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<td>100</td>
<td>165</td>
</tr>
<tr>
<td>Arsenate</td>
<td>$10^{-3}$</td>
<td>100</td>
<td>128</td>
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<tr>
<td>Iodoacetate</td>
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<tr>
<td></td>
<td>$10^{-5}$</td>
<td>5–10</td>
<td>—</td>
</tr>
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<td></td>
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<td>100</td>
<td>37</td>
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<tr>
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<td>0–20</td>
</tr>
<tr>
<td>Phosphate</td>
<td>$2 \times 10^{-3}$</td>
<td>30</td>
<td>—</td>
</tr>
</tbody>
</table>

(e) Effect of Borate, Phosphate, and Magnesium Ions

Borate ion (as boric acid, $5 \times 10^{-4}$M and $1 \times 10^{-3}$M) and phosphate ion (as potassium dihydrogen phosphate, $2 \times 10^{-3}$M) both failed to stimulate sugar accumulation in sugar-cane tissues, instead causing a reduction (Fig. 7; Table 1). Magnesium ion (as magnesium chloride, $2 \times 10^{-3}$M and $5 \times 10^{-3}$M) caused a 0–20 per cent. increase in the rate of sugar accumulation (Fig. 7). The concentration of borate used may have been slightly toxic to sugar-cane. Alternatively, there could have been an optimal concentration of boron already present in the control tissue. This was probably the case for magnesium and phosphate.
IV. DISCUSSION

The effect of the various inhibitors on the sugar-accumulation process in sugar-cane conformed closely to the general pattern of action of inhibitors on many other transport processes in plant and animal tissues. Compared with these, the sugar-accumulation process in sugar-cane was generally sensitive to lower inhibitor concentrations than most plant processes, but less sensitive to inhibitors than the sugar-accumulation process in animal tissues. However, the only major difference found concerned the action of phloridzin. This inhibitor at $10^{-6}-10^{-5}M$ has been shown by a number of workers to be a specific inhibitor of sugar accumulation in animal tissues: in sugar-cane there was no effect at concentrations as high as $10^{-4}M$, and even at $2 \times 10^{-3}M$ inhibition was not complete. At this concentration phloridzin is believed to act as a general inhibitor of phosphorylation (James 1953a). It is possible that no effect was observed at low concentrations because the tissue inactivated the phloridzin. If this were so, however, it would be expected that when dilute solutions of the inhibitor were used, there would be an initial phase when inhibition occurred (as with cyanide, Fig. 5). At higher concentrations the pattern was rather one of increasing inhibition with time. This is more what would be expected if the inhibitor, being of high molecular weight, was hindered in reaching the site of action in the tissue. In view of the rapid action of the other inhibitors, in particular PCMB which also has a high molecular weight, this too seems unlikely. Weatherley (1953) found that phloridzin at a concentration of 0·1 per cent. ($2 \times 10^{-3}M$) did not inhibit sugar uptake in leaf tissues; though there is some doubt as to whether this author was studying a true accumulation process (Pennell and Weatherley 1958). Street and Lowe (1950) found that growth of tomato roots was

![Graph](image-url)
inhibited 25 per cent. by $2 \times 10^{-4}$M and 67 per cent. by $2 \times 10^{-3}$M phloridzin and that the inhibition was partially reversed by increased sucrose concentrations. They attributed the inhibition to an effect of phloridzin on sucrose utilization. Thimann and Marre (1954) found that phloridzin, $2 \times 10^{-3}$M, inhibited the elongation of *Avena* coleoptiles, but the inhibition was independent of sugar uptake, and not reversed by the addition of hexose phosphates. In all these cases the effective concentration was high and the degree of inhibition slight as compared with phloridzin inhibition of sugar uptake in animal tissues. It appears that the sugar-accumulation process in plants may differ from that in animals by not being specifically sensitive to phloridzin.

![Graph](image)

**Fig. 7.**—Effects of magnesium ion and borate ion on sugar accumulation in immature tissues: ○ 0.275 per cent. glucose; △ 0.275 per cent. glucose + $2 \times 10^{-3}$M magnesium chloride. □ 0.275 per cent. glucose + $5 \times 10^{-4}$M boric acid. Tissue prewashed for 21 hr in distilled water, $2 \times 10^{-3}$M magnesium chloride, and $1 \times 10^{-3}$M boric acid respectively. Tissue sample weight 65 g; solution volume 130 ml.

It was found that, in general, when inhibitors were added to the tissue, there was either a very slight inhibition of sugar accumulation, or a complete inhibition and a rapid sugar leakage from the tissue. In some cases the inhibition and leakage could be stopped and accumulation restored by removal of the inhibitor. It is therefore unlikely that the sugar leakage was always, if ever, due to any destruction of cell membranes by the inhibitor. There could have been a sudden increase in the permeability of the tonoplast once a critical inhibitor concentration was reached. However, three distinct types of inhibitors were involved; inhibitors of aerobic respiration (anaerobic conditions (Bieleski 1960), cyanide), inhibitors of phosphate transfer (DNP, arsenate), and inhibitors which act on enzymes containing -SH groups (PCMB, cupric ion, mercuric ion), each of which stopped sugar accumulation and caused sugar leakage when present in a concentration similar to that in which it usually acts on most other processes in plant tissues (James 1953a, 1953b).
Hence it is unlikely that the sugar leakage was caused by a direct effect of inhibitors on the cell permeability. The evidence suggests that, in the immature storage tissues at least, net leakage of sugar from the vacuole is prevented only by the countering action of an accumulation mechanism which obtains energy from the normal metabolic processes of the cell. Once the inhibitor is present in amounts sufficient to inhibit this accumulation mechanism, sugars pass freely from the cell into the outside solution. The degree of activity of the accumulation system determines whether sugar will be taken into the tissue, held at equilibrium, or lost by leakage. The behaviour of the mature tissue is more uncertain. Anaerobic conditions have been shown to cause sugar leakage in immature tissues, but apparently not in mature tissues, despite the greater concentration of sugars (Bieleski 1960). This suggests that as the cell reaches final maturity it becomes more impermeable and less dependent on the accumulation mechanism for maintaining its internal sugar concentration. Some of the results with DNP inhibition supported this suggestion, since sometimes DNP inhibition resulted in a slower leakage from mature tissue than from immature tissue (e.g. Fig. 2), but occasionally (e.g. Fig. 3) the reverse was found. Variations in time of year, duration of the experiment, and concentration of DNP all affected the relative leakage rates from the two tissues; and the tissue itself apparently modified the action of the inhibitor by slowly inactivating the DNP. Clearly, other methods will have to be sought in order to determine whether or not changes in tonoplast permeability occur in the maturing sugar-cane storage cells.

V. Acknowledgments

The author wishes to thank Dr. R. N. Robertson and Professor F. V. Mercer, Plant Physiology Unit, C.S.I.R.O., and the Botany School, University of Sydney, for advice and encouragement; Professor R. L. Crocker, Botany School, University of Sydney, in whose Laboratories this work was carried out; and the Colonial Sugar Refining Co. Ltd. and their employees for their help and care in supplying and delivering the sugar-cane material. These studies were made during the tenure of a C.S.I.R.O. Research Studentship.

VI. References


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